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BIOLOGICAL BULLETIN

OF THE

Marine Biological Laboratory

WOODS HOLE, MASS.

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BIOLOGICAL BULLETIN

THE RELATIVE PERMEABILITY OF THE SURFACE AND INTERIOR PORTIONS OF THE CYTOPLASM OF ANIMAL AND PLANT CELLS.[1]

(A PRELIMINARY PAPER.)

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The permeability and osmotic properties of cells are explained on the assumption of the presence of surface and vacuolar plasmatic membranes which are of a semi-permeable or partially permeable character. It is generally held that the remainder of the protoplasm of a given cell plays a negligible rôle in both diosmosis and permeability. These assumptions are based on indirect evidence. The permeability of the internal cytoplasm of living animal and plant cells has not been investigated by direct methods.

This paper gives the results of a study of the permeability of the internal cytoplasm and nucleus to dyes and crystalloids.

The animal material used in this investigation included the eggs of *Asterias*, *Cumingia*, *Chætopterus*, *Nereis* and the immature eggs of *Necturus*, *Ameba proteus*, *Paramecium* and the striped muscle and epidermal cells of *Necturus*. The plants selected were *Saccharomyces*, *Mucor*, *Saprolegnia*, some five species of *Spirogyra*, *Hydrodictyon*, the manubrial cells of *Chara*, the leaves of *Elodea*, root-hairs of *Vicia faba*, *Pisum*, *Hordeum* and the parenchyma cells of *Tradescantia*.

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[1] The studies on marine eggs reported in this paper were carried on during the summer of 1912 at the Marine Biological Laboratory, Woods Hole, Mass., while occupying a table through the courtesy of the Director, Dr. F. R. Lillie, to whom I am indebted for many kindnesses.

A number of methods were used in order to make the data comparative and to determine the error and limitations of a given method employed. The effect of various operations, such as partial dissections, and punctures, on the permeability and osmotic properties of cells was studied. Intracellular injections were made by Barber's method.[1]

THE PERMEABILITY OF ANIMAL AND PLANT CELLS TO ACID DYES.

Incidental to this study, a number of facts bearing on Overton's so-called lipoid theory have been determined, and as animal physiologists have taken kindly to this notion some of these data will be given.

Rhuland[2] and others have shown a number of exceptions to Overton's[3] conclusion that cells are impermeable to lipoid insoluble acid dyes.

By using a large number of species of animals and plants from widely separated genera and phyla, I have found it quite easy to discover cells that are freely permeable to many lipoid-insoluble acid dyes. Such well-known acid dyes as eosin and trypan red are good vital stains for *Mucor* and *Saprolegnia*.

The lipoid-insoluble acid dyes used include:

Trypan blue, which penetrates the eggs of *Nereis* and *Chætopterus*, the root-hairs of barley and the Windsor bean, immature *Necturus* eggs, the peritoneal epithelium of *Necturus*, *Ameba proteus*, *Paramecium*, *Mucor*, and *Saprolegnia*.

Trypan red: the eggs of *Cumingia* and *Chætopterus*, the root-hairs of barley, the edible pea and the Windsor bean, *Ameba proteus*, *Paramecium*, *Mucor*, and *Saprolegnia*.

Isamin blue: the root-hairs of barley and the Windsor bean.

Analine blue: the eggs of *Chætopterus*, the root-hairs of barley and the Windsor bean, and *Mucor*.

Acid fuchsin: The root-hairs of barley, the Windsor bean and *Mucor*.

Acid green: *Saprolegnia* and *Mucor*.

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[1] Barber, *Jour. of Inf. Dis.*, 1911, VIII., p. 348.

[2] *Jahr. Wiss. Bot.*, Bd. 51, H. 3, p. 376.

[3] *Jahr. f. Wiss. Bot.*, Bd. 34, 1900, p. 669.

Acid violet: Windsor bean root-hairs, *Saprolegnia*, and *Mucor*.

Beibricher scarlet: Barley root-hairs, the immature eggs of *Necturus*, the peritoneal epithelium of *Necturus*, *Mucor*, *Saprolegnia*, and *Paramecium*.

Indigo-carmin: *Saprolegnia* and *Mucor*.

Ponceau, P. R.: the root-hairs of the Windsor bean, *Ameba proteus*, *Paramecium*, *Saprolegnia*, and *Mucor*.

Indulin: root-hairs of Windsor bean, *Ameba proteus*, *Saprolegnia*, and *Mucor*.

Nigrosin: the peritoneal epithelium and the very immature eggs of *Necturus*, and *Mucor*.

Eosin: Windsor bean root-hairs, *Ameba proteus*, *Paramecium* *Mucor*, and *Saprolegnia*.

In recent papers Loewe[1] states that even basic dyes are adsorbed by lipoids, when added to solutions of lipoids in organic solvents.

THE INTRACELLULAR INJECTION OF DYES AND CRYSTALLOIDS.

If indigo-carmin, methyl red, trypan blue, thiocarmine R., or azolitmin dissolved in sea-water be injected into any portion of the cytoplasm of the starfish egg, the sea water slowly diffuses into the surrounding protoplasmic gel, and finally the granular precipitated dye alone remains. An injection of indigo-carmin into the cytoplasm of an immature egg of *Necturus* results in a slight staining of the wall of the vacuole, while an injection of such widely different dyes, as indigo-carmin, trypan blue, and janus green (diethyl-safranin-azo-dimethyl-anilin), into the cytoplasm of the striped muscle cell of this animal results at most in a localized staining of the cytoplasmic gel immediately surrounding the mass of injected dye. The injection of indigo-carmin or nigrosin into the vacuole of *Spirogyra* results in a blue-green or light violet staining, respectively, of the entire cytoplasm. A blue-green staining of the cytoplasm of *Hydrodictyon* is affected by an intravacuolar injection of indigo-carmin. In fact, every acid dye that was injected into the vacuole of *Spirogyra*, *Hydrodictyon*, the leaf cells of *Elodea*, and the parenchyma cells of *Tradescantia* penetrated, and stained the cytoplasm and usually the nucleus.

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[1] Loewe, *Biochemische Zeitschrift*, 1912, XLII., p. 150.

Particularly striking permeability phenomena are to be observed when dyes that do not penetrate *Ameba proteus* are injected into the interior. Azolitmin, congo red, tropeolin 000 No. 1, sodium alizarin sulphonate, and indigo-carmin were used for these injections.

All the dyes enumerated were dissolved in salt and sugar solutions of different concentrations, and small and large doses were injected. It was found that these dyes diffuse quickly through the interior of *A. proteus*, if the concentration of the salt or sugar solution be not too high. A localized blue vacuole results when indigo-carmin, dissolved in distilled water, is injected into the ectoplasm of this animal. Usually in a short time the vacuole breaks into the interior and the dye rapidly diffuses.

Sea water, distilled water, solutions of sodium chloride, potassium nitrate, and cane sugar have been injected into the interior of different types of cells. A small dose of distilled water is taken up by the surrounding cytoplasm of the starfish egg quite slowly. A vacuole of sea water requires a somewhat longer time to disappear, while a vacuole filled with hypertonic sea water increases in size. Hence, any portion of the cytoplasm of the starfish egg can exhibit the same general diosmotic properties that are shown by the surface. It seems that this is also true for the cytoplasm of the striped muscle cell of *Necturus*, but on account of the very high viscosity of this substance the wall surrounding the injected fluid remains very irregular, and no accurate measurement of the volume injected could be made. Distilled water is absorbed from the interior of the muscle substance extremely slowly; and salt and sugar solutions more slowly or not at all.

Doses of 1 m. sodium chloride and potassium nitrate and from .5 M. to 2 M. cane sugar diffuse through the cytoplasm of *Ameba proteus* when injected into the interior. The vacuoles that are formed by the injected fluid quickly collapse. Granules, fibrils, and globules can be produced in *proteus* by the injection of 2 M. cane sugar. The shrunken part of the cytoplasm does not readily take up water again.

In this connection it may be added that the injection of mercury and isotonic salt solutions and the introduction of various foreign bodies into protoplasm usually lead to remarkably little reaction.

THE EFFECT OF CHEMICAL TREATMENT AND OPERATIONS ON THE PERMEABILITY OF CELLS.

Early in this investigation a general relation between permeability and the degree of concentration of protoplasmic gels was noted. Several methods were devised for grading the concentration of the surface and interior portions of the cytoplasm of various cells. The desired concentration gradient can be produced in the eggs of *Asterias*, *Chætopterus*, *Cumingia*, and *Nereis* either by treating with very dilute acids, alkalies and saponin dissolved in sea water or puncturing or cutting with extremely fine Jena glass needles. It was proved that such operative treatment does not kill any portion of the egg.

If the egg of *Asterias* be punctured, the acid dyes used penetrate the swollen area for varying depths, but never enter the normal unswollen cytoplasm. Under the conditions of my experiments only the swollen surface was penetrated and stained. It cannot be overemphasized that the concentration gradient experiment has proved an adequate test of the correctness of the membrane conception, at least for the eggs of *Asterias*, *Cumingia*, *Chætopterus* and *Nereis*. Dyes were selected which do not penetrate the normal egg. These dyes penetrate the swollen cytoplasm produced by the operation or chemical treatment, to varying depths, but never enter the unswollen cytoplasm. Some of the acid dyes do not penetrate the surface of the swollen area of a punctured egg, while others are stopped only by the unchanged cytoplasm. These results are exactly the converse of what would necessarily follow if the plasmatic membrane conception were really true. Moreover, it was noted that trypan red and erythrosin stain the surface of a normal *Chætopterus* egg, but even a great increase in concentration of the dyes does not produce more than a surface staining. Here the surface is actually more permeable to these dyes than the interior of the egg. Again, the nucleus of the eggs of *Asterias*, *Cumingia*, *Chætopterus*, and *Nereis* remain unstained when the eggs are placed in the best vital stains in use at the present time. Under this set of conditions the nuclear membrane is impermeable to vital stains. This structure is a concentrated tough gel of relatively high viscosity and is not to be confused with hypothetical surface or vacuolar plasmatic membranes. If the concentration of a suitable vital stain be raised considerably or the nucleus be dissected out, staining occurs.

Puncture of the walls and probably the cytoplasm of various types of plant cells has given unanticipated results. All the more common acid dyes enter the cells of such plants as *Spirogyra*, *Elodea*, *Hydrodictyon*, different root-hairs, and the parenchyma cells of *Tradescantia*, following an extremely small puncture. Puncture of the walls of slightly plasmolyzed *Spirogyra* and *Elodea* cells is followed by protoplasmic staining when acid dyes are added. On the other hand, puncture of thoroughly plasmolyzed *Spirogyra* cells is followed at most by a slight surface staining, of the shrunken and concentrated cytoplasm, by the same acid dyes.

During plasmolysis of *Spirogyra* a large amount of mucilaginous material is poured out of its wall, and, as a result, this structure loses much of its rigidity, becomes softer, and permeable to many acid dyes. This striking change can be demonstrated by vital stains, dissection of the wall of thoroughly plasmolyzed cells, and by staining cells immediately after recovery from thorough plasmolysis.

A very small cut in the wall of a manubrial cell of *Chara* gives even with trypan blue and trypan red only a local staining of the cytoplasm, which slowly spreads.

The Dissection of Cells.

The chief cells that have formed the material for this study have been dissected by the use of adequate methods, and their physical properties, such as rigidity, viscosity, glutinicity, elasticity, tenacity, and colloidal state, have been determined. Although these data are too extensive to be given here, they form a part of the basis of my conclusions.

Conclusions.

1. The structural components of protoplasm vary greatly in their permeability to water, dyes, and crystalloids.

2. Impermeability or partial permeability to water, dyes, and crystalloids is a property of all portions of protoplasmic gels.

3. The rate of penetration of protoplasm by dyes and crystalloids is, in general, inversely proportional to the concentration of the living gel.

4. The best vital stains known penetrate such highly concentrated protoplasm as the epithelial and striped muscle cells of *Necturus* very slowly.

5. The interior portions of the cytoplasm of the starfish egg and probably the striped muscle cell of *Necturus* exhibit the same sort of osmotic properties as the surface.

6. The cell-walls, and not the protoplasm of many plant cells, prevent the entrance of dyes.

SPERMATOGENESIS OF THE PIG WITH SPECIAL REFERENCE TO THE ACCESSORY CHROMOSOMES.

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Introduction.

In late years the occurrence of accessory chromosomes, in various degrees of complexity, has been recorded from diverse groups of invertebrates. Recently the field has been extended to the vertebrates, Guyer having shown their existence in the males of the guinea ('09*a*), rooster ('09*b*), rat ('10), and man ('10); Newman and Patterson ('10) in the armadillo; Jordan ('11) in the opossum; Stevens ('11) in the guinea-pig; and King ('12) in *Necturus*. The existence of what appears to be such an element has also been reported in the ovary of the cat by Winiwarter and Saintmont ('09). In a recent paper Jordan ('13) states that the heterochromosomes are unquestionably lacking in the pig. I find, however, that the accessory chromosomes are very conspicuous in all of the pig material which I have studied.

Wilson ('09) found in *Syromastes* that half of the spermatids possess two more chromosomes than the remainder. It was predicted by him that in consequence the somatic cells of the female of this species would show two more chromosomes than the somatic cells of the male, and later the facts were found to be in exact accord with his predictions, the somatic cells of the female of *Syromastes* having been found to contain twenty-four, those of the male, twenty-two chromosomes. A similar condition has been found in other tracheates, but dimorphism in the number of chromosomes in the germinal and somatic cells of the two sexes among the vertebrates has thus far been only inferred, not actually demonstrated.

The present study on the spermatogenesis of the pig was taken up at the suggestion of Professor M. F. Guyer, to whom I am much indebted for many helpful suggestions during the progress of the work. Points of special interest in this paper are as follows:

1. The presence of a distinct pair of accessory chromosomes.
2. The resulting dimorphic condition in the spermatozoa of the pig.
3. Dimorphism in the number of chromosomes in both the germinal and somatic cells of the male and female animals.
4. The abundance of large conspicuous interstitial cells in the testes.
5. The second reduction of the chromosome number in the secondary spermatocyte, which was found to be simply equational.
6. The throwing off of a large mass of cytoplasm, containing one of the centrosomes, at the time of the final development of the spermatozoan.

MATERIAL AND METHODS.

I have been very fortunate in obtaining from several sources exceptionally good material for this investigation. The major part of the material studied was obtained from a vigorous Poland China boar about ten months old. The animal came from registered stock and was the property of the College of Agriculture of the University of Wisconsin. The material was obtained through the courtesy of Professor L. J. Cole. Immediately after the testes were removed from the live animal, pieces were placed in various kinds of fixing fluids, as Bouin's, Zenker's, Tellyesnicky's, and corrosive-acetic.

Sections from various parts of the testes were made from four to twelve microns thick and while several methods of staining were employed, material fixed in Bouin's fluid and stained with Heidenhain's iron hematoxylin with acid fuchsin as a counterstain, proved to be the most satisfactory. Delafield's hematoxylin with eosin counterstain was also used and gave favorable results. While material fixed in some of the other fluids mentioned was fairly good, the first method proved to be so superior that the sections used in this study were almost wholly those prepared according to it. Other material, from a much older animal, was studied in sufficient detail to corroborate the results obtained through the study of the material secured from the younger and more vigorous animal.

I also owe many thanks to Professor B. M. Allen for his generosity in placing at my disposal his embryological material of pigs of both sexes. The material in question, which was that used by Dr. Allen ('04) in his own researches on the embryology and development of the ovary and testes in mammals, was fixed in Flemming's fluid and stained with Heidenhain's iron hematoxylin mainly. By examining a large number of these slides, I not only corroborated my previous count of the spermatogonial number of chromosomes, but was also able to make fairly conclusive counts of the chromosomes in the somatic cells of the male, and in the oögonia and somatic cells of the female. Without the use of his material this important phase of the problem could not have been worked out at this time.

General Arrangement of the Germinal Cells.

The structure of the testes of the pig does not differ greatly from that of other well-known mammals, except for the presence of numerous masses of large well-defined interstitial cells which are scattered among the seminiferous tubules and comprise about one fourth of the entire volume of the testes (Fig. 1). A detailed description of these interesting cells, which contain numerous mitochrondia, will be reserved for a separate paper. The germinal cells themselves are found in a great number of seminiferous tubules coiled throughout the interior of the testes. Sections of the tubules are found in groups of fifteen to twenty-five which are completely surrounded by walls of connective tissue. This connective tissue forms a sort of continuous network of walls throughout the testes and is rather thick in places, usually where three or four of the groups become appressed. Embedded in these walls are blood- and lymph-vessels which branch out into the masses of interstitial cells between the tubules.

The arrangement of the cells in the tubules is similar to that of the other warm-blooded animals and the usual four types of germinal cells are present. The spermatogonia form a more or less regular layer of cells lying next to the wall of the tubule.

These divide and give rise to two new cells, one or both of which may become the primary spermatocyte. The primary spermatocytes are very abundant, especially in the spireme stage. After various changes and considerable growth the primary spermatocytes divide and give rise to the secondary spermatocytes. The secondary spermatocytes divide in turn to form the spermatids, which transform directly into spermatozoa (Figs. 64–78).

The material studied must have been in maximum activity at the time of fixation, for the four types of germinal cells were easily found in stages of growth and development. Mitotic stages of spermatogonia, primary and secondary spermatocytes, were very abundant, and frequently the entire field under the oil immersion lens was composed of cells in mitosis.

Spermatozoa in all stages of development were very abundant, and clusters of the cells which were in the final stages of development could be seen attached to the long cylindrical Sertoli cells which often extend to the lumen of the tubule (Figs. 2 and 3). The Sertoli cells, which are quite abundant, are closely connected with the germinal cells and will be described in more detail later (Fig. 63).

Spermatogonia.

The spermatogonia usually lie in a single layer next to the wall of the tubule though occasionally some of the cells are crowded out, thus forming a second layer which is always very irregular. There is considerable variation in the appearance and some variation in the size of different spermatogonia. Very frequently the cells are far apart, in which case they are flattened out on the tubule wall.

The amount of cytoplasm is small and in some of the earlier stages the cell boundaries are very indistinct (Fig. 16). Later the nuclei assume a round shape and the cell wall becomes visible. The amount of chromatin material increases and the nuclei at this stage resemble very much the nuclei of the large interstitial cells both in appearance and size (Fig. 17).

Two large nucleoli much alike in size seem to be present in all stages of these cells. Even in the testes of young embryos the two bodies are very conspicuous. Besides these large nucleoli a varied number of smaller, similarly staining bodies are usually present, two of which seem to occur most frequently, and evidently make their appearance first (Figs. 16 and 17). Later other small nucleoli appear. At present I am unable to attach any meaning to them as their numbers are so varied in the various cells. The two large nucleoli, however, are very constant, and I have been able to trace them throughout the entire spermatogenesis. From all appearances one is led to believe that these nucleoli and the accessory chromosomes are one and the same, a condition similar to that found by Guyer ('10) in man, and by others in some of the lower forms. Jordan ('11), however, has not been able to identify any such structures at this stage as the future accessory chromosomes in the opossum.

At the conclusion of the resting stage numerous chromatin granules appear, which arrange themselves along fine threads in an entangled mass. The two nucleoli come in close contact and the nuclear membrane gradually disintegrates. Eighteen chromosomes appear in the late prophase of the spermatogonial division (Fig. 18). Sixteen of these are rod-shaped, variously curved and somewhat different in size. Two, which are always found close together, and frequently off to one side are slightly larger and oval in shape. The sixteen ordinary chromosomse or autosomes arrange themselves with the two accessory chromosomes in the equatorial plate (Fig. 19). The accessories, like the autosomes, divide in this stage. A centrosphere containing a tiny centrosome can be observed in some cases, but its presence is not at discernible in these early cells as it is in the later stages.

PRIMARY SPERMATOCYTES.

1. *Resting Stage.*

The primary spermatocytes which arise from the final spermatogonial division in the early resting stage are usually somewhat smaller than the spermatogonia in the metaphase and prophase stages. The two large nucleoli are quite conspicuous and remain black in sections stained with iron hematoxylin even in very much destained material (Fig. 20). Usually two small nucleoli, stained the same way as the large ones, are plainly visible and occasionally only one, or more than two of these bodies appear.

2. *Synizesis and Growth Period.*

An increase in the bulk of both the nucleus and cytoplasm begins after a brief period of rest. The chromatin appears to be arranged in much the same way as it was during the resting condition of the spermatogonia, except that the linin fibers are coarser. The cytoplasm is composed of granular masses and clear, apparently liquid areas. The chromatin threads and nucleoli become massed at one side of the nucleus (Fig. 21). The nuclear wall expands and the clear area formed by the massing of the chromatin to one side becomes much enlarged. The clear areas in the cytoplasm decrease coincidentally with the increase of the liquid-like area in the nucleus and one is led to believe that the cytoplasmic fluid permeates the nuclear wall during the period of synizesis.

During the collapse of the chromatin material, the nucleoli can be plainly seen especially in well destained sections (Fig. 21). They are usually in that portion of the chromatin mass which is nearest to the nuclear wall. Opposite this mass on the outside of the nuclear wall a centrosphere can often be seen, but the centrosome is rarely visible at this stage. The chromatin threads become arranged in a very much tangled mass of loops which later appear in about half the original number and fully twice as thick. (I have not been able to determine definitely whether the pairing takes place by telo- or parasynapsis.) The whole mass then moves toward the center (Fig. 22), the large clear area in the nucleus disappears, and the nuclear wall becomes spherical and clearly defined (Fig. 23). While the formation of the spireme is going on, the cytoplasm, too, increases greatly in volume and appears very granular. The cell now is fully twice as large as a spermatogonium and judging by the large number of cells that can be seen in the spireme stage one can safely conclude that they remain in that condition for some time. The spireme finally breaks up into U and variously shaped chromosomes (Fig. 25). The two large nucleoli, which remain in full view throughout this stage, become oblong and can often be seen close together (Fig. 25).

3. *Reduction Division.*

The primary spermatocytes when ready for division reveal ten chromosomes in the late prophase or early metaphase stage. The two accessories which are ordinarily off to one side (Figs. 8, 26, 27, and 29) can be recognized at a glance. The other chromosomes are usually arranged in a ring. Judging from their large size and changed form, they are bivalent, representing the paired univalent chromosomes of the spermatogonium. That is, of the original eighteen chromosomes, sixteen have paired to form eight bivalents of the primary spermatocyte and two have remained unpaired as the accessory chromosomes. Sometimes, although ten chromosomes can be counted, it is difficult to tell just which are the accessories owing to overlapping. The chromosomes differ somewhat in size as can be seen in Figs. 26 to 29. Figs. 9 to 12 and 30 to 37 show the accessories in characteristic positions in the metaphases of division of the primary spermatocyte. They always pass entire, side by side and in advance of the divided autosomes, toward one pole. This is possibly due to the fact that they are not retarded by division.

The fact that the chromosomes immediately after divergence (Fig. 28) resume the appearance (except in size) that characterizes the univalent spermatogonial chromosomes, and also because the accessory chromosomes pass over entire to one pole in this division while they are halved in the next division, seems to indicate strongly that this is the reduction division.

It is obvious that as far as chromatin content is concerned the division of the primary spermatocyte gives rise to two dissimilar cells, one of which receives eight chromosomes, and the other eight plus the two accessories or ten chromosomes (Fig. 38). Figure 39 is a drawing of one end of a late anaphase of such a division showing eight chromosomes, and Fig. 40 shows the other end which received eight of the ordinary chromosomes and the double accessory.

Occasionally a small chromatin body is present in this first spermatocytic division (Figs. 28, 31, 32, 35 and 37). Figure 31 shows such a body passing to the same pole with the accessories, in advance of the other chromosomes. Figure 32 represents an earlier stage of much the same thing. In Fig. 35 it can be seen passing to the opposite pole, and Fig. 37 represents an extremely rare case where two such bodies are present, one somewhat larger, passing to either pole, even in advance of the two accessory chromosomes. Figure 28 shows the body outside of the main ring of chromosomes. While the small body can be seen frequently, as a rule no such an element can be detected, and while it may possibly be comparable to the small pair of chromosomes found so constantly in some of the Tracheata, my present data on its irregular occurrence and behavior do not permit a conclusion regarding its significance.

Secondary Spermatocyte.

1. *Dimorphism.*

The dimorphism of the secondary spermatocytes, which resulted from the last division, is again expressed in the resting stage that sometimes follows. Approximately half of them showed, under proper decolorization, two large chromatin nucleoli (Fig. 41) while in the others only the small nucleoli appeared (Fig. 42). The nucleoli retain the usual deep staining capacity, and as was true in the previous stages, even when all the other material is almost totally decolorized, the nucleoli remain very conspicuous. Frequently, both primary and secondary spermatocytes were found dividing in the same field, which fact seems to suggest that at times there is no intervening period of rest between the two divisions, or that it is very brief. Figure 43 shows the two resulting cells of a primary spermatocyte division which are still in close contact and both ready for dividing into spermatids. Resting stages, however, appear in abundance and in this stage, as was the case in the resting stage of the primary spermatocyte, the centrosome surrounded by a clear zone becomes large and very conspicuous (Figs. 41 and 42).

2. *Second Reduction Division* (*Equational*).

Although the division of the primary spermatocyte gave rise to cells containing eight and ten chromosomes respectively (Figs 38, 39, 40), when these cells become ready for division half of them show four (Figs. 13, 14, 43, 45, 46) and the other half six chromosomes (Figs. 43, 44, 47). Thus a second pairing of the ordinary chromosomes, similar to that found by Guyer in the pigeon ('00), corroborated by Geoffrey Smith ('12), man ('10), guinea ('09*a*), and chicken ('09*b*), and by Jordan in opossum ('11), has evidently taken place so that there are four bivalents in each type of cell and the additional two accessory chromosomes in the one type. Stevens ('11) says that there is no such second synapsis or numerical reduction in the guinea-pig. Figures 45 and 46 show four large chromosomes in the metaphase stage of one type of secondary spermatocyte and Figs. 44 and 47 represent the other type in which six chromosomes appear, four bivalent autosomes plus the two accessories. The four chromosome group is evidently formed by the pairing of the eight chromosomes of one type of cell resulting from the first maturation division at one pole, and six chromosome group is interpreted as being derived by the pairing of the eight chromosomes plus the two unpaired accessories at the opposite pole (Figs. 38, 39, 40).

Guyer ('10) in speaking of the second conjugation of the chromosomes in man says:

"Assuming that the respective chromosomes are more or less qualitatively differentiated, such a numerical reduction, however, by no means necessarily implies that there has also been a second qualitative reduction. Aside from the improbability of such a reduction, the general appearance of the divided chromosomes would not warrant this interpretation; for instead of the elongated univalent type as seen in the spermatogonia, or in anaphases of the divisions of spermatocytes of the first order, the daughter chromosomes here retain the rounded appearance and increased size that is characteristic of the bivalent types (compare Figs. 1, 10, and 11, 13, 14, 15, 16 and 17). Thus while half of the spermatids receive five, and half seven chromosomes, in terms of univalence the numbers would in all probability be ten and twelve respectively."

Jordan ('11) in speaking of the same condition in the opossum remarks as follows:

"Similarity of form between the chromosomes of the first and second metaphase plates (*i. e.*, double rods) suggests a similar manner of division: accordingly a second reduction. When one recalls, however, that a resting stage (Figs. 39 to 41) usually intervenes between the first and second maturation divisions, when the chromosomes pass through a reticular phase, the above conclusion is inadmissible; or rather, no definite conclusion respecting the character of the second division is justified. A double true reduction, suggested by the form of the chromosomes is contrary to our fundamental conceptions regarding the significance of chromosomes and need not, in view of the nature of the evidence, be seriously considered. Moreover, in the stage just preceding the brief resting phase of the spermatids (Figs. 57, 58 and 59) there occurs a resolution of the five chromosomes into nine and of the four into eight. This demonstrates that the true character of the second division is equational. The second numerical reduction involves a less close union apparently than the first, as a comparison of illustrations 29 and 43 will show. Again the fusion is sometimes incomplete to the extent of giving an occasional count of six chromosomes."

My own belief that the real character of the second division in the case of the pig is simply equational is based on a number of facts. Even after the first maturation division the ordinary chromosomes are much larger than the spermatogonial univalents. This increase in size has evidently taken place during the growth period of the primary spermatocyte (compare Figs. 18 and 38). During the prophase of the secondary spermatocyte the chromosomes apparently increase still more in size and four large bivalent autosomes appear for division in the one type of cell and four plus the two accessories in the other type. Not only is the bivalent nature of these large autosomes conspicuous, but very frequently a quadrivalent character is discernible and they are much larger than the accessories (Figs. 44, 45, 46 and 47). During the anaphase the bivalent nature of the chromosomes is often clearly visible at each pole. Figure 49 shows four bivalents and is a drawing of one pole of the dividing cell which received eight chromosomes during the first maturation division. Figure 52 is a drawing of one pole of the division of the type of secondary spermatocyte which received eight ordinary chromosomes and the double accessory. It will be seen that four of these are bivalent in nature, while two are univalent, the two univalents being the results of the division of the two accessories (Figs. 44, 50 and 51) both of which have here divided for the first time since the spermatogonial division. The bivalent nature of the autosomes after the second spermatocytic division becomes even more conspicuous in cases where these chromosomes divide into two before they break up. Figure 56 shows an early spermatid cell which received the four bivalent autosomes and the two accessories. It can be seen that two of the autosomes have almost completely divided. The bivalent nature of the other two can also be plainly seen, while the accessories retain their univalent appearance. Thus it can be seen that the spermatids produced by this second division do not receive four and six chromosomes respectively, but, four bivalents, or the equivalent of eight univalents, are present in the one type of cell, and four bivalents or eight univalents together with the two accessories in the other. The foregoing facts seem to indicate beyond doubt that the second maturation division is not a reduction but simply an equational one.

Figures 50 and 54 represent an anaphase of division in a secondary spermatocyte showing two streaks of lagging chromatic material. Although several such cases were observed ordinarily no such pronounced streaks occur (Fig. 51). Guyer found a similar condition in the secondary spermatocyte division in man and suggests that it may be the two accessories lagging behind. Although many accurate counts of the chromosomes in this second division were made, they were so massed together as to render a count impossible (Fig. 54). Not infrequently the chromosomes in the early spermatids become closely appressed before they begin to break up (Figs. 53 and 57). Sometimes the individuality of the chromosomes is apparent even after the nuclear wall begins to form (Fig. 58).

SPERMATIDS.

From the foregoing evidence it is obvious that there exists a dimorphism among the spermatids, one type containing eight and the other ten chromosomes after the last division. The chromosomes soon become irregular in shape and begin to break up, and the spermatids appear all alike except that immediately after the chromosomes disintegrate two nucleoli are visible in approximately half of the cells (Figs. 64 and 65). These retain for a short time the staining capacity characteristic of the nucleoli of the previous stages, but later disappear and the whole nucleus assumes a coarse granular appearance. The cells seemingly remain quiescent in that condition for some time as they are nearly always found in large numbers. The centrosome surrounded by a clear layer is again very conspicuous within the comparatively small centrosphere which lies close to the nucleus (Figs. 64 and 65).

DEVELOPMENT OF THE SPERMATOZOA.

Following the period of rest the spermatids begin to develop into spermatozoa. There is no perceptible change in the size of any of the parts, such as described by Jordan in the opossum. The first change to be detected is the extrusion of the centrosome, surrounded by a light area, from the small sphere (Fig. 66). It takes a position a short distance from the nucleus and soon begins to divide. The two new centrosomes move apart and one, somewhat rod-shaped, comes in contact with the nuclear wall but it remains connected by a mass of material with the other centrosome which assumes a disk shape and for a while remains in place (Figs. 67, 68 and 69). The disk is frequently perforated in the middle and has the appearance of a ring (Fig. 70).

Simultaneously with the division of the centrosome, a portion of the sphere which remains in close contact with the nuclear wall migrates to a point directly opposite the centrosome in contact with the wall on the other side (Figs. 66, 67 and 68). The sphere in this process of migration is in such close contact with the nuclear wall that a slight depression can be detected in the latter. The depression is even more pronounced when the clear sphere becomes definitely fixed as the small acrosome (Figs. 68–75).

In the guinea-pig the acrosome, which becomes nearly as large as the nucleus itself, according to Meves ('99), is also formed from the centrosphere or idiozome. While in the rat, according to Lenhossek, it is independently formed in the cytoplasm without relation to the preceding mitotic figure or the centrosomes. Meves ('97) also found that in the salamander the acrosome is formed from the idiozome which wanders around the nucleus to its anterior pole. McGregor's results on *Amphiuma* ('99) agree in general with those of Meves, except that here the acrosome arises from only a part of the centrosphere, while a second smaller part passes to the base of the nucleus and forms the main part of the middle-piece. Coincidentally with the division of the centrosome and the migration of the small sphere the nucleus together with these structures moves to one side of the cell in the direction of the acrosome and soon practically all of the cytoplasm is at the posterior end of the cell (Figs. 66, 67, 68, 69 and 70). The cell wall seems to persist as a thin mantle covering the head of the spermatozoan.

The anterior part of the cell, bearing the acrosome, almost invariably points in the direction of the tubule wall while the mass of cytoplasm extends into the lumen (Fig. 2). Most of the chromatic material of the nucleus gathers at the center into a dense mass which has the same staining capacity as characterizes the chromosomes and the centrosome. Sometimes two or three masses are present (Figs. 73, 74 and 75).

Soon after the division of the centrosome into a cylindrical anterior and a disc-shaped posterior body, a divergence of the two follows, but they remain connected by a streak of material (Fig. 69). The inner body upon coming in contact with the nuclear wall forms a depression in the latter and its anterior portion shapes into a disc which comes in close contact with the nuclear wall in the small depression at the posterior end of the nucleus. From this disc, which is apparently the end-knob, a cylindrical mass of material extends backwards uniting the two centrosomes (Figs. 68 and 69). A tiny filament extending backward from the middle of the posterior disc-shaped centrosome makes its appearance and is undoubtedly a continuation of the coarser filament which unites the two centrosomes (Fig. 69). As the tail grows longer the connecting filament becomes thinner, and the posterior centrosome, which after division was disc-shaped and had increased somewhat in size, becomes transformed into a ring and it can be seen that the filament extends directly through it and continues backward as the tail (Fig. 70). The formation of the ring takes place simultaneously with the rapid growth of the tail and one is led to believe that the perforation in the disc is partly due to the fact that the material formerly occupying that space goes to help in building up the axial filament. Shortly after the tail projects out of the cell the ring moves along the filament, backward, and soon swerves over to one side in the cytoplasmic mass (Figs. 71 and 72). Very frequently it can be seen a considerable distance away from the axial filament long before the latter is fully developed (Figs. 73, 74 and 75). This seems to indicate that the posterior centrosome takes no further part in the development of the filament and that the latter is mainly developed from the anterior centrosome, no part of which is discarded or thrown off. While most of the inner centrosome passes into the formation of the axial filament a part of it remains as the end-knob in the small middle-piece (Figs. 76 and 78), a condition similar to that found by McGregor in *Amphiuma* ('99).

The posterior ring-shaped centrosome, after moving away from the filament, sometimes divides (Fig. 73), but usually assumes a spherical shape (Fig. 74) and an interesting point in connection with this body is that during the final development of the spermatozoan it is invariably thrown off with a big mass of cytoplasm (Figs. 74, 75 and 76). The casting off of a portion of the cytoplasm during the last stages of the developing spermatozoan has been described by Meves ('99) in the guinea-pig where it is closely similar to the process which occurs in the spermatozoid-formation in ferns; but the throwing off of a portion of the centrosome together with the mass of cytoplasm has not heretofore been, to my knowledge, recorded. The body usually stains as deeply as it does in the earlier stages for some time after the comparatively large mass of cytoplasm containing it is completely separated from the rest of the cell or young spermatozoan.

In general, the behavior of the centrosomes in the development of the spermatozoan of the pig does not differ greatly from the conditions found in some of the other vertebrates. However, it might be well to briefly point out some of the differences. In the spermatids of the salamander, according to Meves ('97), the two centrosomes lie quite at the periphery of the cell and from the outer one grows out the axial filament. The two centrosomes leaving the idiozome by which they are first surrounded, now pass inwards toward the nucleus, the outer one meanwhile becoming transformed into a ring while the axial filament passes through it to become attached to the inner centrosome. The latter pushes into the base of the nucleus and enlarges enormously to form a cylindrical body comprising the main portion of the middle-piece. The ring divides into two parts, the anterior of which gives rise to a small body at the posterior end of the middle-piece identical with the end-knob. The other part of the ring wanders out along the tail and finally lies at the limit between the main part of the latter and the end-piece.

In the pigeon, according to Guyer ('00), the centrosome divides and moves out of the sphere and further away from the nucleus. The two new centrosomes move apart, but remain connected by a mass of material which later disappears and a very delicate fibril uniting the two centrosomes exists in its place. One of the centrosomes enlarges and transforms into a complete ring. The connecting fibril can later be seen passing from the smaller centrosome back through the ring and outside the cell. The centrosomes next approach the nucleus and as they draw near a slight invagination appears in the nuclear wall and the small centrosome moves into it.

In the mammals, the work of Lenhossek on the rat ('98) and Meves on the rat, guinea-pig and man ('98, '99) gives a result similar to the condition found in the salamander. In all these mammals the young spermatids contain two peripherally placed centrosomes, from the outer one of which the axial filament grows out and the centrosomes later move toward the nucleus. In the pig the centrosomes are never peripherally located and division of the spermatid centrosome does not take place until it emerges, surrounded by a small clear sphere, from the main bulk of the centrosphere which is in close contact with the nucleus.

It can be seen from the drawings of the later stages that the nucleus gradually elongates and flattens (Figs. 67–78). The large chromatic mass within the nucleus disintegrates and the particles become distributed at the periphery of the nuclear wall (Fig. 78). The tail envelope becomes apparent as the cell elongates and evidently develops from the cytoplasm (Figs. 74–78). It later comes in contact with the axial filament and envelops about half of its entire length. The remaining extremely thin portion of the tail is apparently the naked axial filament (Figs. 76, 77, 78).

When the cells reach the stage represented in Figs. 71–73 they attach themselves in bunches to the large cylindrical Sertoli or nurse cells that often extend from the basement membrane a long distance toward the lumen of the tubule (Figs. 2 and 63). As the spermatozoa continue to develop a gradual decrease in the volume of the cytoplasmic mass of the Sertoli cells is noticeable. When the spermatozoa are apparently mature they abandon the Sertoli cells which at this time are very much collapsed. The spermatozoa after leaving the nurse cells do not pass directly to the lumen of the tubule, but remain scattered for some time among the masses of cytoplasm which were cast off by the same developing cells a short time before (Figs. 3 and 67). This cast-off material does not scatter about in the lumen, as one would suspect, but forms a sort of loose layer next to the cells following the maturing spermatozoa. The masses begin to disintegrate soon after they are discarded by the developing spermatozoan, and when stained with iron-hæmatoxylin numerous black bodies make their appearance in this material (Fig. 3). The spermatozoa remain, with their bodies embedded in this layer, and tails extending into the lumen, until the mass almost completely disappears. Then they become free in lumen of the tubule and are ready to make their way out of the testes. During this period of attachment to the nurse cells and later to the discarded mass of cytoplasm the heads of the spermatozoa increase somewhat in size but retain the same staining capacity. The foregoing facts seem to show beyond doubt that the developing spermatozoan derives nourishment not only from the nurse cells but also from the cast-off cytoplasmic material. When the sperm reaches maturity the acrosome disappears from view and the anterior edge of the head becomes well rounded.

In addition to the parts mentioned, there appears very frequently a dense spherical cytoplasmic mass represented in Figs. 67 to 75. As to the origin and fate of this body I am not entirely certain. It seems, however, that it is a portion of the centrosphere, for in many of the early stages it was seen in the immediate neighborhood of the small body which later forms the acrosome (Fig. 67). Further evidence for this assumption is the fact that the combined mass of these two bodies seems to be equal to the size of the sphere in the stage immediately preceding (Fig. 66). The consistency of this body, too, is similar to that of the sphere.

Figure 78 represents a mature spermatozoan of the pig which appears rather simple in structure and form. The entire nucleus of the spermatid has evidently developed into the head, which is oblong and flat (Figs. 70–78). The nuclear material breaks up into very fine particles which arrange themselves in a layer at the entire periphery of the head wall (Fig. 77).

With Heidenhain's iron-hæmatoxylin the head stains a sort of slate blue and is difficult to decolorize. A depression is present at the posterior extremity which is in contact with a small middle piece. The end-knob which is for some time attached to the nuclear wall within the small depression (Figs. 74, 75 and 76) finally breaks loose and passes to the posterior extremity of the middle piece, the greatest portion of which now remains very clear in appearance. This clear area is similar in appearance to the contents of the acrosome and one is led to believe that it is due to the presence of the substance which was seen in form of a clear sphere surrounding the centrosome after it emerged from the centrosphere and persisted about the two centrosomes, particularly around the inner one, after division (Figs. 66–69)

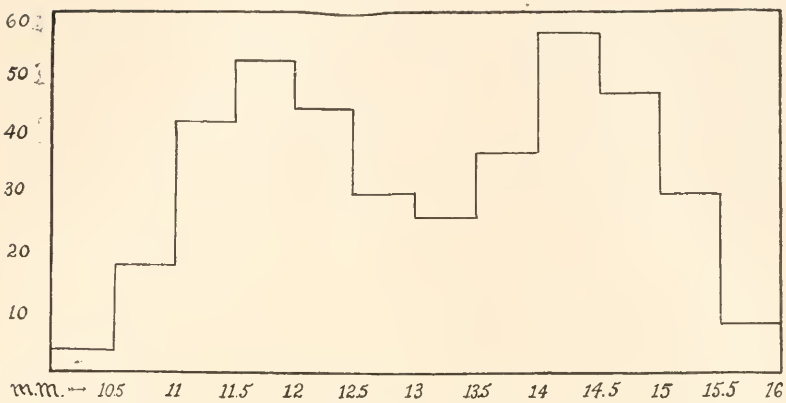

FIG. 1. Diagram showing the variation in size among four hundred mature pig spermatozoa. Figures at the left give the numbers of the individuals belonging to each type. Figures at the bottom give the lengths of the heads of the spermatozoa in millimeters, magnified two thousand times.

VARIATION IN SIZE OF MATURE SPERMATOZOA.

The spermatozoa of the pig vary considerably in size, and careful measurements revealed the fact that they may be arranged in two separate classes, one type being much larger than the other. Mature specimens, which were free in the lumen of the tubule and parallel to the objective, were selected at random, and outline sketches of four hundred heads enlarged (× 2,000) were made with the aid of a camera-lucida. The lengths of the sketches were then carefully measured and recorded in half millimeters. Figure 1 in the text shows the variation in size of the four hundred heads measured. It can be seen at a glance that two separate types of spermatozoa exist; the greatest number of the one kind measuring from 11.5 to 12 mm., and of the other type from 14 to 14.5 mm. The increased size of the latter is due presumably to the presence of the accessory chromosomes.

RELATION OF THE ACCESSORY CHROMOSOMES TO SEX IN THE PIG.

Many investigators working on the problem of sex determination are of the opinion that the sex of a zygote is determined at the time of fertilization, the spermatozoan in most cases carrying the determining factor. This view has been recently substantiated by the careful investigations of McClung, Stevens, Wilson, Morgan, Payne and others, who found that in many invertebrates such as certain insects, myriopods, arachnids and nematodes, two different kinds of spermatozoa are produced. The spermatozoa differ from each other by one chromosome, or by a group of chromosomes, called the accessory chromosomes, or X-chromosomes.

In some forms it was found that the spermatozoa each contain an accessory chromosome, but that in half the sperms this chromosome was larger in its chromatin content than in the other. These are called the X and Y elements respectively. All the eggs produced (except in a few cases), on the other hand, contain the X-element.

It has been shown in some of the Tracheata that whenever an egg is fertilized by a sperm containing the X-chromosome it develops into a female, while an egg fertilized by a sperm without the X-element, or by one containing the Y-element, gives rise to a male individual.

In the vertebrates, Guyer was able to identify an X-element in such diverse forms as the guinea, rooster, pigeon, rat, and man, and other investigators have recently brought forth similar evidence. Patterson and Newman ('10) record its presence in the armadillo, Jordan ('11) in the opossum, Stevens ('11) in the guinea-pig, and King ('12) in *Necturus*. Up to the present investigation, however, a dimorphism in the number of the chromosomes in the male and female vertebrates has not been recorded. Guyer ('10) after speaking of the difference in the number of chromosomes in the somatic cells of the male and female Tracheata which possess the X-elements says:

"In the light of these facts we should expect the somatic cells of man to contain twenty-two and of woman, twenty-four chromosomes. The tissues of the female have not yet been studied with this in mind. Flemming ('97) records the somatic number of chromosomes, determined from corneal cells, as twenty-four but unfortunately he does not record the sex of the subjects from which the material was obtained. If it were a female his count would bear out the interpretation given above."

The behavior of the accessory chromosomes in the various stages of the spermatogenesis of the pig further substantiates the fact that two different kind of spermatozoa are produced in mammals. Since the dimorphic condition of the spermatozoa does exist, the question arose whether this dimorphism holds true in the chromosome number of the male and female somatic cells. With this in view I undertook an examination of sections of male and female embryological material supplied me by Professor B. M. Allen. A large number of these sections were studied and although mitotic stages were not very abundant in any one section, and the chromosomes were frequently bunched together rendering a count impossible, nevertheless, a number of counts of the chromosomes in the germinal and somatic cells of the two sexes were recorded. It was again found that the spermatogonial number of chromosomes is eighteen, and that the same number prevails in the somatic cells found in the mesonephros of the male embryos. Two of the chromosomes are usually somewhat larger (Fig. 59). Sections of the female material revealed a count of twenty chromosomes in the oögonia (Fig. 60), and the same number prevails in the somatic cells found in the mesonephros of the female embryos (Fig. 62). Four large chromosomes corresponding to the two accessories in the male can usually be detected in the oögonial cells. In the somatic cells of the female there are also four large chromosomes present corresponding to the two accessories in the male cells and the four accessories in the oögonia (Fig. 62).

In a few cases ten large chromosomes were found in the early metaphases of division in the somatic cells of the female (Fig. 61). Two of these are considerably larger and are interpreted as the result of the pairing of the four accessories. The smaller chromosomes, eight in number, judging by their size, are evidently due to the pairing of the sixteen other chromosomes. The position of these cells would not warrant the supposition that they might be wandering germ cells. Furthermore, a similar condition was not observed among the oögonial cells in spite of the fact that mitotic stages occur far more frequently in the sections of the ovaries than they do in the mesonephros.

In proportion to the large number of mitotic stages found in these long-continued searches, accurate counts of chromosomes in the cells of the various tissues were comparatively few. However, all cases where a count was possible were recorded and although they varied somewhat, the above number of chromosomes, eighteen and twenty, attributed to the male and female cells respectively, were the prevailing numbers.

The foregoing facts are in accord with the expectations, and we have here in a vertebrate a condition verifying the results found in some of the lower forms. In view of these facts it is obvious that the eggs, carrying ten chromosomes, or half the somatic number, when fertilized by a spermatozoan containing ten chromosomes, give rise to an individual containing twenty chromosomes in its cells, or a female. Those fertilized by the other type of spermatozoan, which contains only eight chromosomes, give rise to individuals with eighteen chromosomes in their cells, which was found to be true in the male.

The results of the present investigation, therefore, add support to the chromosome theory of sex determination, since they show that in the vertebrates, as well as in some of the lower forms, there exists a dimorphism in the number of chromosomes in the somatic as well as the germinal cells of the two sexes. It is highly probable that conditions similar to those found in the pig, as regards sex determination, exist in man and in the other vertebrates which possess the accessory or X-chromosomes. The resemblance in the behavior of the pair of accessories in the pig and their behavior in man is very striking and suggests that in all probabilities there exists a dimorphism in the germinal and somatic cells of man and woman.

Summary.

1. The usual four types of cells, the spermatogonia, primary and secondary spermatocytes, and spermatids are discernible in the spermatogenesis of the pig.

2. Large interstitial cells containing numerous mitochondria exist in great abundance and comprise about one fourth of the entire mass of the testes.

3. Numerous Sertoli or nurse cells are present and great numbers of spermatozoa can be seen in all stages of development in the various tubules.

4. Two large nucleoli are present in the resting stages of the spermatogonia. These can be traced through the entire spermatogenesis of the pig and are apparently correlated with, or are the same thing as the two accessories. Smaller nucleoli, usually two, are also present.

5. Eighteen rod-shaped chromosomes differing somewhat in size occur in all of the spermatogonia where a definite count could be made. Two of these, undoubtedly the accessories, can usually be seen to one side of the main mass of chromosomes.

6. During the spermatogonial division the accessories divide and occasionally pass to the poles in advance of the other chromosomes.

7. The last spermatogonial division gives rise to cells which through a process of growth become the primary spermatocytes. Both nucleus and cytoplasm increase greatly in size. Synizesis followed by synapsis occurs.

8. An apparently continuous spireme is formed which later breaks up into U-shaped and variously curved chromosomes. A centrosphere containing the centrosome is present.

9. The two large round nucleoli which remain very conspicuous throughout the process of growth of the primary spermatocyte come together and elongate during the late prophase.

10. Ten chromosomes appear for division in the primary spermatocyte of which eight are evidently bivalent and two accessory. The accessories are usually considerably to one side of the other chromosome.

11. The two accessory chromosomes which are out of the main spindle pass undivided to one pole in advance of the other chromosomes.

12. The primary spermatocyte division is evidently the reduction division. It gives rise to two cells, one of which contains ten (eight autosomes plus the two accessories) and the other only eight chromosomes.

13. The chromosomes in these daughter cells are larger than the univalents of the spermatogonia and show some indications of bivalence.

14. The secondary spermatocytes formed by the last division ordinarily go into a resting stage. In half of these the two

nucleoli are conspicuous and lacking in the others. In a few cases, at least, there is no resting period in the secondary spermatocytes. A small centrosphere containing a relatively large centrosome is present.

15. During the metaphase of the secondary spermatocyte one half show four large bivalent chromosomes, and the remaining show the four large bivalents plus the two accessories or six chromosomes. A second pairing has apparently taken place but the division is simply equational as the four large chromosomes often manifest a quadrivalent character. The accessories remain unpaired.

16. The quadrivalent nature of the autosomes in the secondary spermatocytes becomes all the more certain after division, as the chromosomes passing to the poles are of the bivalent nature.

17. The type of secondary spermatocyte which received eight chromosomes after the first maturation division gives rise to two spermatids each containing four bivalent or eight univalent chromosomes. The other type which received ten chromosomes after the first maturation division gives rise to two spermatids, each containing four bivalent or eight univalent chromosomes and the two accessories, each accessory having divided here for the first time since the spermatogonial division.

18. The dimorphic nature of the spermatids which develop into spermatozoa is further evinced by the presence of the two nucleoli in approximately half the cells. The centrosome is again conspicuous.

19. The first noticeable change in the transformation of the spermatid is in the centrosome. It emerges from the small sphere and divides into an anterior rod-shaped and a posterior disc- or ring-shaped body.

20. Both of the centrosomes contribute to the development of the axial filament. A portion of the anterior one persists as the end-knob, while the posterior body is finally cast off.

21. A portion of the sphere migrates to the opposite side and gives rise to the acrosome which disappears when the spermatozoan is fully developed.

22. The nucleus of the spermatid passes to one side of the cell, elongates and flattens and forms the head of the spermatozoan.

23. The axial envelope which extends a little more than half the length of the tail is developed from the cytoplasm.

24. The half developed spermatozoa attach themselves to Sertoli cells where they continue to develop.

25. The cytoplasmic mass of the Sertoli cells decreases greatly in size as the spermatozoa continue to develop. Finally, when the spermatozoa are almost mature, a collapse of the nurse cells takes place when practically nothing but the nucleus and the cell wall remain.

26. An interesting event occurs in the final stages of the development of the spermatozoan when a large mass of cytoplasm together with the posterior centrosome is thrown off by the cell.

27. When the spermatozoa desert the Sertoli cells they do not pass directly into the lumen of the tubule but remain scattered with their heads embedded in the layer of the cast-off cytoplasm.

28. The cast-off cytoplasmic material is apparently used as food by the maturing spermatozoa and when practically all of the former disappears the fully developed spermatozoa become free in the lumen of the tubule.

29. Measurements of the mature spermatozoa reveal the fact that they are of two distinct sizes, a point no doubt correlated with the presence and absence of the accessory chromosomes.

30. The somatic cells of the male contain eighteen chromosomes, a number corresponding to that in the spermatogonia.

31. The somatic cells of the female yield a count of twenty chromosomes as do the oögonia.

32. The foregoing facts add considerably to the support of the chromosome theory of sex determination, since they prove that in the vertebrates, as is true of some invertebrates, there exists a dimorphism in the germinal and somatic cells of the male and female.

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EXPLANATION OF PLATES.

Plate I.

(Fig. 1, ×200; Figs. 2 and 3, ×500; Figs. 4–12, ×1,200.)

1. Section of a pig testis showing the seminiferous tubules separated by wide areas of interstitial cells.

2. Section of a tubule showing bunches of developing spermatozoa attached to Sertoli or nurse cells.

3. Section of a tubule showing almost fully developed spermatozoa which have deserted the Sertoli cells and became scattered among the masses of cast-off cytoplasm.

4. Resting stage of a spermatogonial cell showing two large nucleoli.

5. Primary spermatocyte in the synizesis stage.

6. Resting stage of a primary spermatocyte showing the two large nucleoli.

7. Spireme stage of a primary spermatocyte showing the chromatin threads and the two persisting nucleoli.

8. Late prophase of division in a primary spermatocyte showing eight chromosomes in the main group, and the two accessories lying off to one side. The photograph does not reveal the full size of the accessories.

9 and 10. Side views of primary spermatocytes showing the cell ready for division with the accessories lying just above the level of the regular equatorial plate of chromosomes.

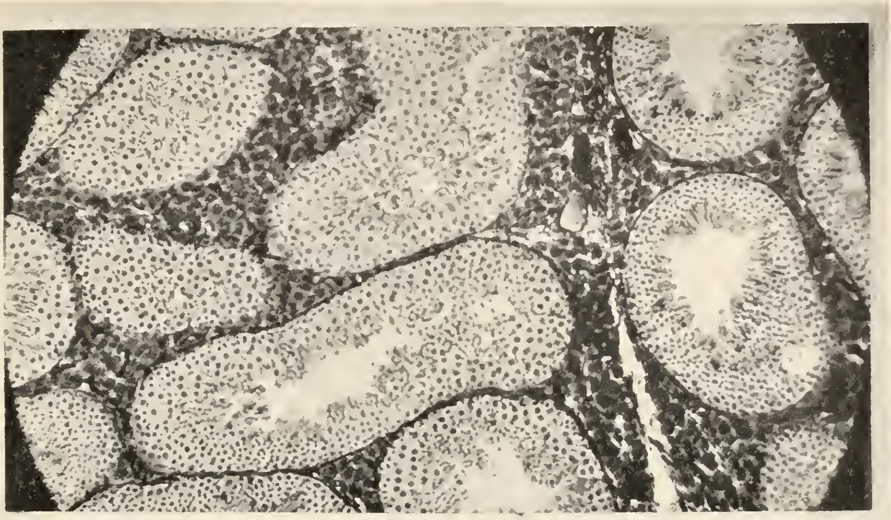
11. Side view of a primary spermatocyte division with the two accessory chromosomes passing to the pole, side by side, in advance of the other chromosomes.

12. Side view of a primary spermatocyte division showing the two accessories at the pole while the autosomes are still in the equatorial plate undivided.

13. Two metaphases of secondary spermatocytes showing four chromosomes in the equatorial plate. The figure at the top is a side view, and the one at the bottom a polar view.

14. The cell at the top is one type of secondary spermatocyte which contains four chromosomes in the metaphase stage and the cell below is a spermatid resulting from the division of a type of secondary spermatocyte which received eight autosomes plus the two accessories.

15. Resting stage of a spermatid showing two nucleoli.



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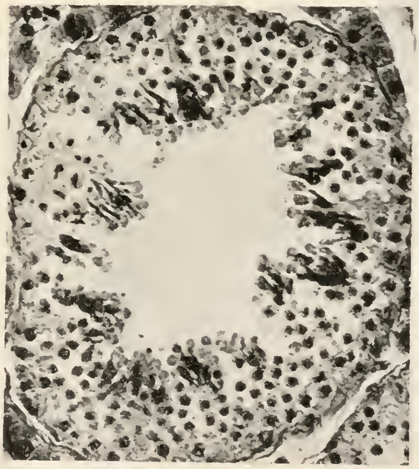

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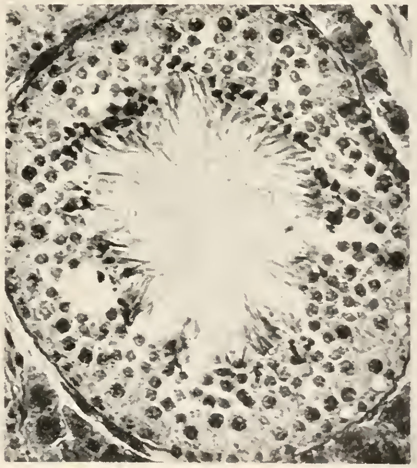

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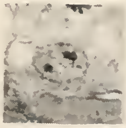

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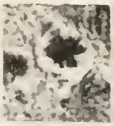

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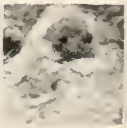

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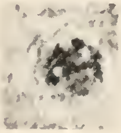

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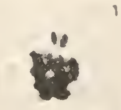

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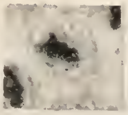

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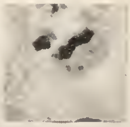

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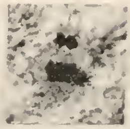

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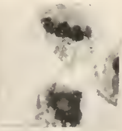

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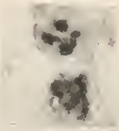

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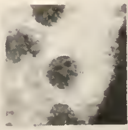

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PLATE II.

(All of the drawings on plates 2–6 were made with the aid of a camera lucida. ×2,330.)

16. Early spermatogonial cell showing two large nucleoli. The cell boundary is quite indistinct.

17. Resting stage of a full grown spermatogonial cell showing two large and two. small nucleoli.

18. Late prophase of a spermatogonial division showing eighteen chromosomes· The two large oval chromosomes at the top are apparently the accessories.

19. Metaphase of division in a spermatogonium showing the two divided accessories at the right.

20. Early resting stage of a primary spermatocyte showing the centrosome, two large, and several small nucleoli.

21. Primary spermatocyte in synizesis.

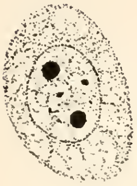
22. Primary spermatocyte following synizesis and synapsis; the entangled mass of threads moved toward the center of the nucleus and the threads appear to be twice as thick as those in the synizesis stage.

23. Primary spermatocyte following the synaptic stage. The clear area in the nucleus disappears and the nuclear wall becomes well developed.

24. Spireme stage of a primary spermatocyte showing increase in size of both the nucleus and the cytoplasm and also the two large nucleoli.

25. Breaking up of the spireme into U and variously shaped chromosomes. The two nucleoli become oval in shape and can always be seen close together in this stage.

26, 27 and 28. Late prophases of primary spermatocytes showing ten chromosomes. The two oval accessories, which are smaller than the bivalent autosomes, are shown in characteristic positions.



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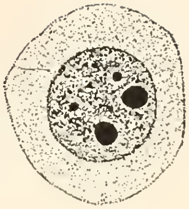

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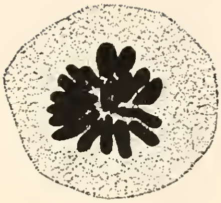

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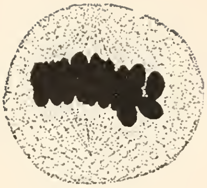

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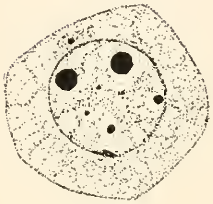

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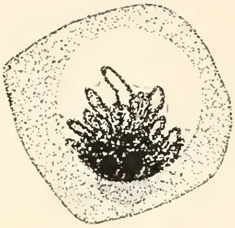

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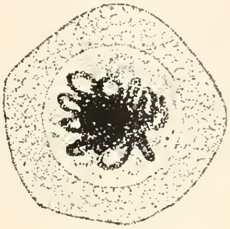

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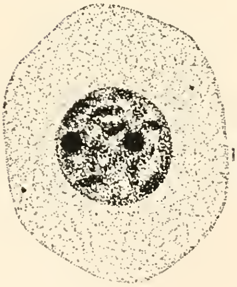

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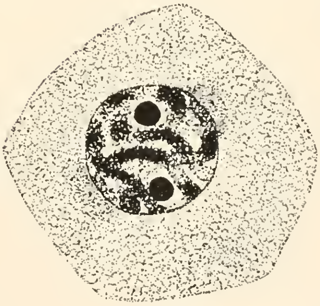

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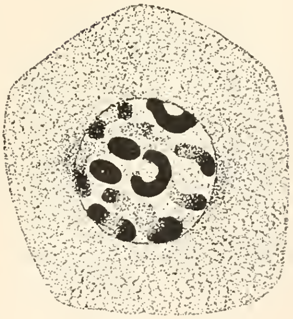

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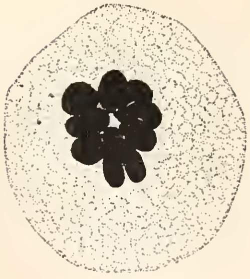

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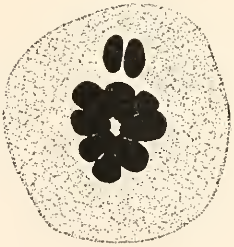

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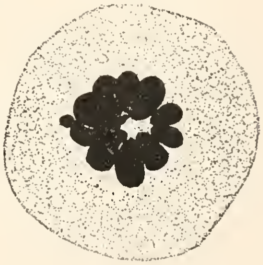

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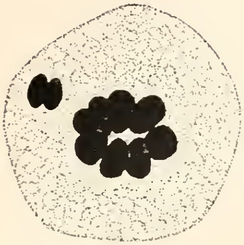
Plate III.

29. Late prophase of a primary spermatocyte showing ten chromosomes, the two accessories lying a considerable distance away from the regular equatorial plate.

30–37. Metaphase of division in primary spermatocytes showing the two accessory chromosomes in characteristic positions passing to the poles. Figures 31 and 32 show also a small body passing to the same pole with the accessories, Figure 35 shows it passing to the opposite pole; and Fig. 37 shows two such bodies, one passing to each pole, which is a case of extremely rare occurrence.



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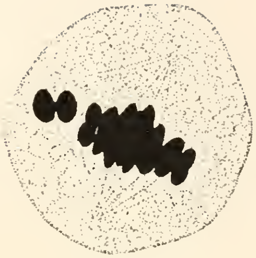

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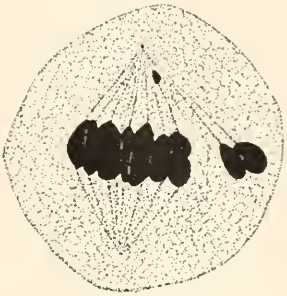

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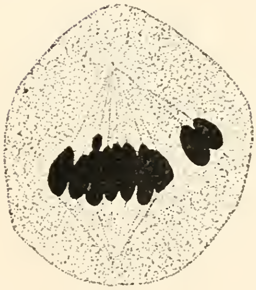

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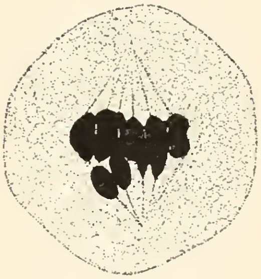

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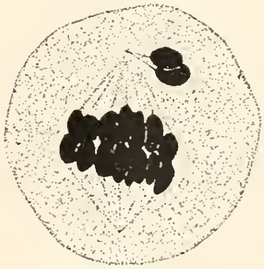

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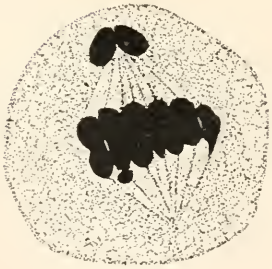

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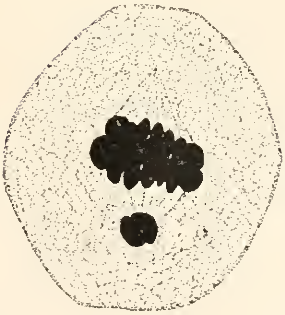

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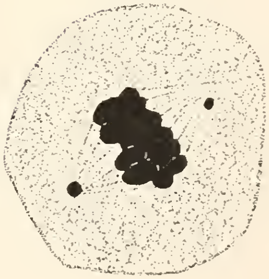

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PLATE IV.

38. Late anaphase of division in a primary spermatocyte showing eight chromosomes at one pole and eight plus the two accessories at the other.

39. One end of a late anaphase of division in a primary spermatocyte showing eight chromosomes.

40. One end of a late anaphase of division in a primary spermatocyte showing ten chromosomes (eight plus the two accessories).

41. Resting stage of a secondary spermatocyte showing two nucleoli and a conspicuous centrosome.

42. Resting stage of a secondary spermatocyte without the large nucleoli.

43. Two contiguous secondary spermatocytes of which one shows four bivalent chromosomes in metaphase of division, and the other four bivalents plus the two accessory chromosomes in metaphase of division. These two cells are no doubt the products of a division of a primary spermatocyte in which eight chromosomes pass to one pole and eight plus the two accessories to the other.

44. Early metaphase of division in a secondary spermatocyte which received the two accessories, showing six chromosomes in all.

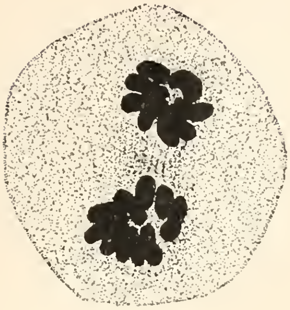
45. Early metaphase of division in a secondary spermatocyte which did not receive the accessories, showing only the four large bivalents.

46. Late prophase of division in a secondary spermatocyte which did not receive the accessories.

47. Late prophase of division in a secondary spermatocyte which received the accessories, showing a total of six chromosomes.

48. Late anaphase of division in a secondary spermatocyte which received eight chromosomes, showing four chromosomes at each pole.

49. A spermatid showing four bivalent chromosomes which is apparently one of the resulting cells of the division of a secondary spermatocyte which received eight chromosomes.



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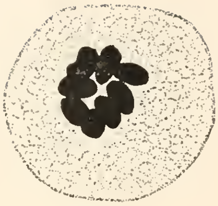

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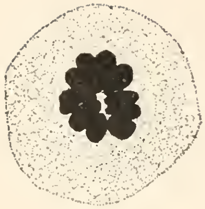

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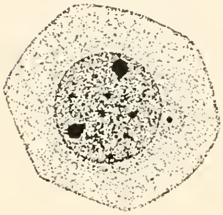

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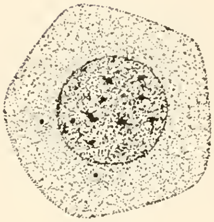

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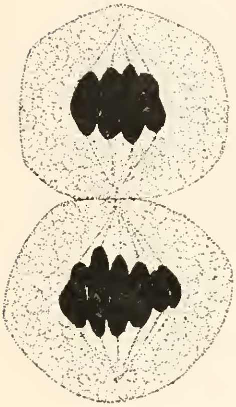

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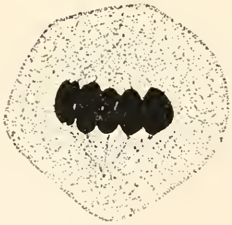

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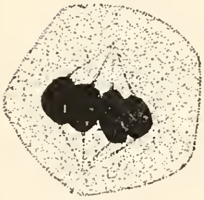

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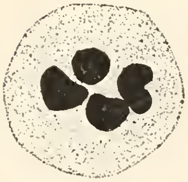

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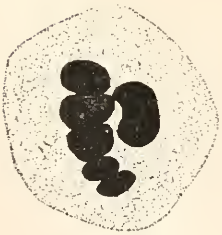

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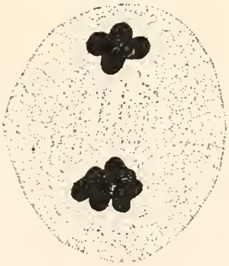

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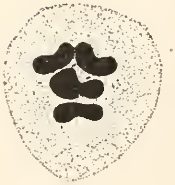

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PLATE V.

50 and 51. Late anaphase of division in a secondary spermatocyte which received the two accessories. Each of the latter divides in this stage. Figure 50 shows streaks of chromatin material uniting the two accessories. Usually no such streaks occur (Fig. 51).

52. One end of a late anaphase of a division in a secondary spermatocyte which received the two accessories. The latter are oval in shape and smaller than the bivalent autosomes.

53. A spermatid which received the accessories, showing a common arrangement of the chromosomes before they break up.

54. Division of a secondary spermatocyte which apparently received the accessories. The chromosomes are so massed as to render a count impossible and the two streaks which rarely occur are probably due to retarded division of the accessories.

55. An early spermatid in which the chromosomes have broken up, while the two nucleoli remain in full evidence and are apparently the same thing as the accessories.

56. An early spermatid, which received the accessories, showing a characteristic breaking up of the bivalent autosomes into univalents. Two of the autosomes had divided almost completely while the other two also show signs of division. The accessories are univalent in nature.

57. A spermatid, which did not receive the accessories, showing a common arrangement of the four bivalent chromosomes. Very frequently these autosomes divide before they become massed together, as is the case of the autosomes in the other type of spermatids represented in Fig. 56.

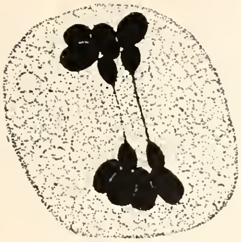
58. A spermatid showing the reconstruction of the nuclear wall while the four bivalent chromosomes are still in full evidence.

59. Late prophase of a somatic cell, found in the mesonephros of a male pig embryo, showing eighteen distinct rod-shaped and oval chromosomes two of which are somewhat larger and are apparently the two accessories.

60. Late prophase of an oögonial division showing twenty chromosomes, four of which are longer and evidently the accessories.

61 and 62. Somatic cells, found in the mesonephros of a female pig embryo. Figure 61 shows a very uncommon condition revealing ten large chromosomes which apparently resulted from the pairing of the twenty chromosomes. Two of these are considerably larger and are interpreted as the result of the pairing of the accessories. Figure 62 shows the late prophase of division in a type of somatic cell occurring most frequently, and reveals a count of twenty chromosomes four of which are considerably larger than the others.

63. A full-grown Sertoli or nurse cell.



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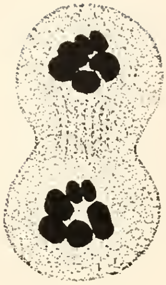

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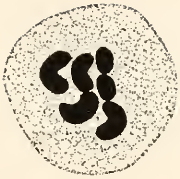

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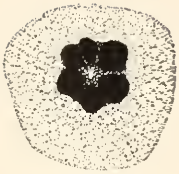

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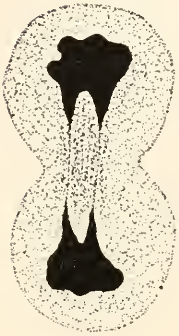

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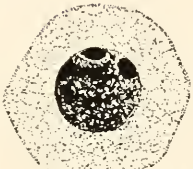

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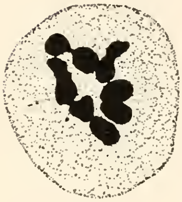

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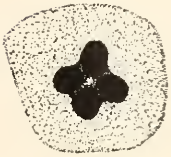

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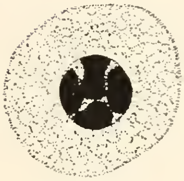

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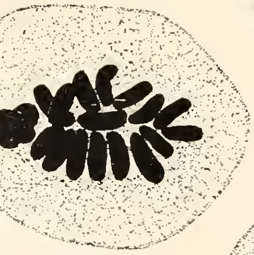

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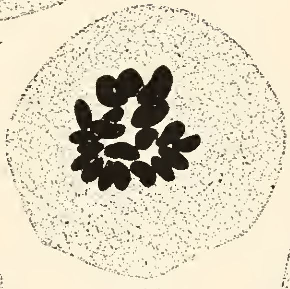

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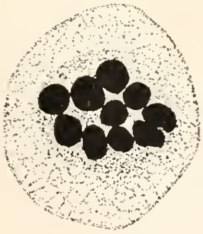

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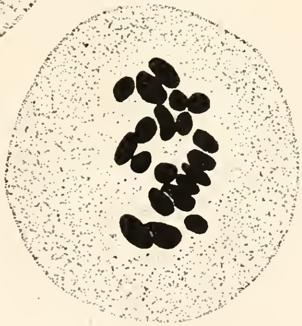

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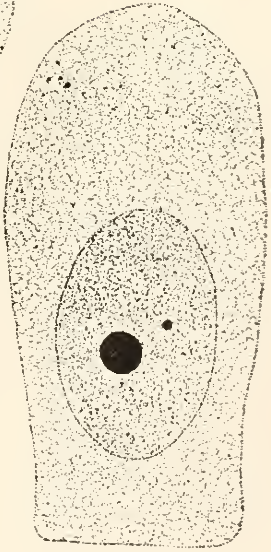

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J. E. WODSEDALEK

Plate VI.

64 and 65. Two contiguous spermatids, one without chromatin nucleoli, the other with two. The spermatids in general are about equally divided into these two classes. The centrosome is very conspicuous in this stage.

66. A spermatid showing the large centrosome, surrounded by a clear area, which had emerged from the sphere a short distance away.

67. A spermatid showing the centrosome in contact with the nuclear wall, and the migration of a portion of the sphere toward the opposite pole.

68. A spermatid showing the division of the centrosome, the position of the sphere as the acrosome, and the migration of the nucleus toward one side of the cell.

69. A transforming spermatid showing the separation of the centrosomes and the beginning of the axial filament. Also a dense spherical cytoplasmic body which is likewise present in some of the following stages.

70. A transforming cell showing a characteristic ring-like structure and position of the posterior centrosome with the axial filament extending through it.

71. A later stage showing the sloughing off of the posterior ring-shaped centrosome from the axial filament.

72. A somewhat later stage showing the posterior centrosome completely separated from the axial filament.

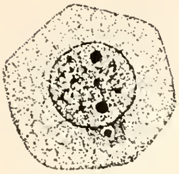
73. A cell showing the cast-off centrosome divided in two.

74 and 75. Still later stages of the developing spermatozoan showing the characteristic position of the posterior centrosome, and the formation of the axial envelope.

76. A spermatozoan almost fully developed showing the mass of sloughed off cytoplasm including the centrosome.

77. Side view of a mature spermatozoan showing the arrangement of the chromatin at the periphery of the head wall.

78. A mature spermatozoan.



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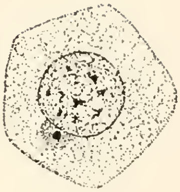

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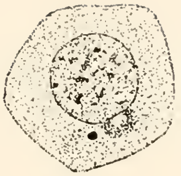

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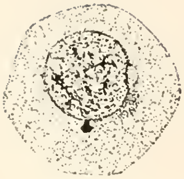

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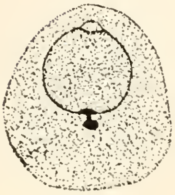

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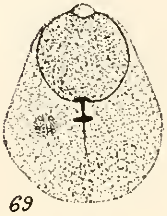

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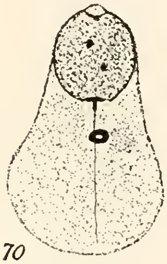

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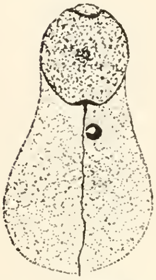

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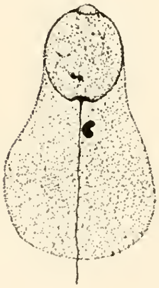

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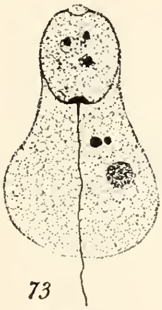

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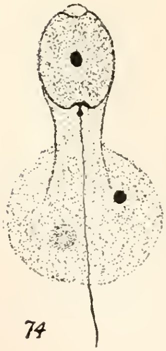

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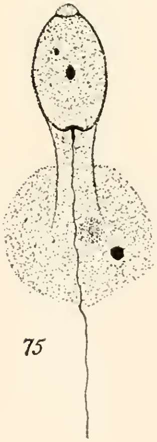

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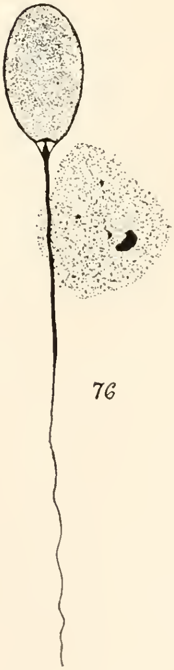

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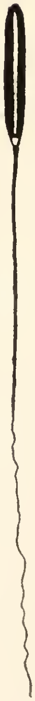

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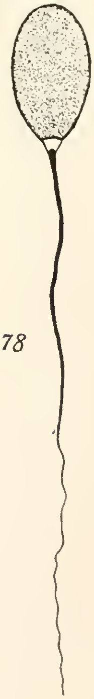

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J. E. WODSEDALEK

A NEW EYE COLOR MUTATION IN DROSOPHILA AND ITS MODE OF INHERITANCE.

SHELLEY R. SAFIR.

A cross between a long-winged, vermilion-eyed female and a miniature-winged, red-eyed male yielded in the first generation red-eyed females and vermilion-eyed males, both long-winged. There appeared, however, in addition 15 long-winged males with a new eye color. This color is of the same general tone as the vermilion pink eye but is paler and somewhat creamier. A lighter area may also be seen encircling the darker area of the center.

The origin of this new eye color was accounted for as follows: Since vermilion is sex-linked, all the sons must be vermilion whatever else they may be. Only males of the new eye color appeared, wherefore it was inferred that the something else added was also sex-linked. There were too many of them (15) to be explained each as a separate mutation, and it seems clear that the mutation itself occurred back in the stock from which the mothers were taken. If one or more of these P_1 vermilion females were heterozygous for the new sex-linked factor we should expect in F_1 several of the double recessive males of the composition cherry vermilion. (It was decided to call the new eye color cherry vermilion.)

If then this eye color represents a double recessive cherry vermilion, when mated to red eye (the normal eye color) there should result in F_1 only red-eyed offspring. Then in F_2, if both of these factors are sex-linked, there should appear four classes of males, viz., the double recessive cherry vermilion, and cherry and vermilion as the result of splitting cherry vermilion into its components, and also red eye color which is the double dominant. All the females should be red. That these results were actually realized can be seen below.

$$\text{red } ♀ \times \text{cherry verm. } ♂ = \left\{\begin{array}{l}\text{red } ♀ \;\; 762 \\ \text{red } ♂ \;\; 758\end{array}\right\rangle \left\{\begin{array}{ll}\text{red } ♀ \ldots\ldots\ldots\ldots & 345 \\ \text{red } ♂ \ldots\ldots\ldots\ldots & 114 \\ \text{cherry } ♂ \ldots\ldots\ldots & 41 \\ \text{verm. } ♂ \ldots\ldots\ldots\ldots & 54 \\ \text{cherry verm. } ♂ \ldots & 120\end{array}\right.$$

The four classes of males taken together number 329, which approximates to the 345 red females. The results may be accounted for in the following way, using the new system of symbols proposed by Morgan (1912).

P_1 red .... ♀ XCV — XCV
cherry verm... ♂ Xcv — ——

F$_1$ Female.
XCV
Xcv
red ♀

F$_1$ Male.
XCV
——
red ♂

Gametes of F$_1$ ♀
XcV — XCV — Xcv — XCv
Gametes of F$_1$ ♂
XCV — ——

F$_2$ Females.		F$_2$ Males.	
XcV XCV	red ♀	XcV ——	cherry ♂
XCV XCV	red ♀	XCV ——	red ♂
Xcv XCV	red ♀	Xcv ——	cherry verm. ♂
XCv XCV	red ♀	XCv ——	verm. ♂

It will be noted that the red-eyed females belong to four classes pure red, reds heterozygous for vermilion, reds heterozygous for cherry, and reds heterozygous for vermilion and cherry. The red and cherry vermilion male classes are each about twice as numerous as the cherry and the vermilion classes respectively. This disturbance in the ratio is due to linkage between cherry and vermilion.

Cherry Vermilion by Vermilion.

When vermilion-eyed females were mated to cherry vermilion-eyed males all the offspring, 483 in number, were vermilion. These when inbred produced vermilion females, vermilion males and cherry vermilion males.

verm. ♀ by cherry verm. ♂ = { verm. ♀ 252, verm. ♂ 231 } > { verm. ♀ ....374, verm. ♂ ....158, cherry verm. ♂ ...158 }

The results bear out the view that the new eye color represented the double recessive; vermilion plus some other sex-linked factor, viz., cherry, as the analysis shows:

P_1 verm. ♀ ....XCv — XCv
cherry verm. ♂ ....Xcv — ——

F₁ Female.
{XCv
{Xcv
verm. ♀

F₁ Male.
XCv
——
verm. ♂

F₂ Females.
XCv / XCv vermilion ♀
Xcv / XCv vermilion ♀

F₂ Males.
XCv / —— vermilion ♂
Xcv / —— cherry verm. ♂

The expectation is as many vermilion as cherry vermilion males and the count shows this is exactly realized.

Cherry Vermilion by Pink.

When pink-eyed females were mated to cherry vermilion males all the offspring, 381 in number, were red. These, when inbred, should produce red, cherry, vermilion, pink, cherry vermilion, cherry pink, vermilion pink and cherry vermilion pink males. Because of difficulty of separation the male classes vermilion pink, cherry pink, cherry vermilion, and cherry vermilion pink were put into one general class. The females were of two classes only, viz., red and pink. The following analysis gives the expectation:

pink ♀ × cherry verm. ♂ = {red ♀, red ♂} >

red ♀	330
pink ♀	79
red ♂	87
cherry ♂	40
verm. ♂	48
pink ♂	29
cherry verm. ♂ cherry pink ♂ verm. pink ♂ cherry verm. pink ♂	126

The eight classes of males taken together number 430 as compared with 409 red and pink females. The results may be accounted for in the following way:

P₁ pink ♀ ....XCVp — XCVp
P₁ cherry verm. ♂ ....XcvP — ——P

F₁ Female.
XCVp
XcvP
red ♀

F₁ Male.
XCVp
——P
red ♂

Gametes of F₁ ♀.
XcVp — XCVp — XcvP — XCvP
XcVP — XCVP — Xcvp — XCvp

Gametes of F₁ ♂.
XCVP — XCVp — ——P — ——p

F₂ FEMALES.	F₂ MALES.
XcVp - XCVP....red ♀	XcVp - ——P....cherry ♂
XcVP - XCVP....red ♀	XcVP - ——P....cherry ♂
XCVp - XCVP....red ♀	XCVp - ——P....red ♂
XCVP - XCVP....red ♀	XCVP - ——P....red ♂
XcvP - XCVP....red ♀	XcvP - ——P....cherry verm. ♂
Xcvp - XCVP....red ♀	Xcvp - ——P....cherry verm. ♂
XCvP - XCVP....red ♀	XCvP - ——P....verm. ♂
XCvp - XCVP....red ♀	XCvp - ——P....verm. ♂
XcVp - XCVp....pink ♀	XcVp - ——p....cherry pink ♂
XcVP - XCVp....red ♀	XcVP - ——p....cherry ♂
XCVp - XCVp....pink ♀	XCVp - ——p....pink ♂
XCVP - XCVp....red ♀	XCVP - ——p....red ♂
XcvP - XCVp....red ♀	XcvP - ——p...cherry verm. ♂
Xcvp - XCVp....pink ♀	Xcvp - ——p...cherry verm. pink ♂
XCvP - XCVp....red ♀	XCvP - ——p....verm. ♂
XCvp - XCVp....pink ♀	XCvp - ——p....verm. pink ♂

It will be noted that the expectation is three red females to one pink female, but the actual count, because of disturbances due to viability, is four red females to one pink female. The male count does not agree with simple expectation in two instances. Both the vermilion and cherry classes which should be as numerous as the red (and the cherry vermilion respectively) are much less numerous than expectation. This, however, is due to coupling between cherry and vermilion.

CHERRY VERMILION BY VERMILION PINK.

When vermilion pink females were mated to cherry vermilion males all the offspring, 635 in number, were vermilion. These when inbred produced vermilion females, vermilion pink females, vermilion males, cherry vermilion males, vermilion pink males and cherry vermilion pink males. Because of difficulty of separation I have included the last two classes in a general class.

verm. pink ♀ × cherry verm. ♂ = { verm. ♀ / verm. ♂ }

verm..............♀	509
verm. pink........♀	208
verm..............♂	223
verm. pink........♂	 89
cherry verm.......♂..	319
verm. pink cherry.♂..	

The four classes of males taken together number 631; the vermilion and the vermilion pink (orange) females number 717.

P₁	verm. pink ♀	XCvp — XCvp
	cherry verm. ♂	XcvP — ——P

F1 Female	F1 Male.
XCvp XcvP verm. ♀	XCvp ——P verm. ♂

Gametes of F1 ♀	XCvp	—	XcvP	—	XCvP	—	Xcvp
Gametes of F1 ♂	XCvp	—	XCvP	—	——P	—	——p

F2 Females.		F2 Males.	
XCvp XCvp	verm. pink ♀	XCvp ——P	vermilion ♂
XcvP XCvp	vermilion ♀	XcvP ——P	cherry verm. ♂
XCvP XCvp	vermilion ♀	XCvP ——P	vermilion ♂
Xcvp XCvp	verm. pink ♀	Xcvp ——P	cherry verm. ♂
XCvp XCvP	vermilion ♀	XCvp ——p	verm. pink ♂
XcvP XCvP	vermilion ♀	XcvP ——p	cherry verm. ♂
XCvP XCvP	vermilion ♀	XCvP ——p	vermilion ♂
Xcvp XCvP	vermilion ♀	Xcvp ——p	cherry verm. pink ♂

The count shows that the vermilion pink females in spite of their low viability ran above expectation. The males fall into four classes, two of which can be easily separated out. The cherry vermilion pinks, however, are difficult to distinguish from the cherry vermilions and are therefore included in that class. Together they should be four times as numerous as the vermilion pink class, and the vermilion class ought to be three times as numerous as the latter, which is actually the case.

Cherry Vermilion by White.

When white-eyed females were mated to cherry vermilion-eyed males all the male offspring the first generation, 363 in number, were white. The females, 392 in number, were cherry. These when inbred produced in the second generation, white females, cherry females, white males, cherry males and cherry vermilion males.

white ♀ by cherry verm. ♂ = { white ♂ 363, cherry ♀ 392 }

white	♀	347
cherry	♀	334
white	♂	321
cherry	♂	112
cherry verm.	♂	222

P_1 white............♀ XcVw — XcVw
cherry verm.......♂ XcvW — ——

F_1 FEMALE.
XcVw
XcvW
cherry ♀

F_1 MALE.
XcVw
——
white ♂

GAMETES OF F_1 ♀ XcVw — XcvW — XcVW — Xcvw
GAMETES OF F_1 ♂ XcVw — ——

F_2 FEMALES.		F_2 MALES.	
XcVw XcVw	white ♀	XcVw ——	white ♂
XcvW XcVw	cherry ♀	XcvW ——	cherry verm. ♂
XcVW XcVw	cherry ♀	XcVW ——	cherry ♂
Xcvw XcVw	white ♀	Xcvw ——	white ♂

The expectation is an equal number of white females and cherry females. The actual count was 347 white females and 334 cherry females. Of the three classes of males the expectation is as many white males as cherry and cherry vermilion put together which is the case as the figures show: 321 white males and 334 cherry and cherry vermilion males. The cherry and cherry vermilion males should be in equal numbers. But this is not the case in the actual count as the cherry vermilion males are twice as numerous as the cherry males: 222 of the former to 112 of the latter. This effect is due to linkage.

CHERRY VERMILION BY EOSIN.

When eosin-eyed females were mated to cherry vermilion-eyed males all the male offspring in the first generation were eosin and the females were cherry. These when inbred produced in the second generation, eosin females, cherry females, eosin males, cherry males, cherry vermilion males, and eosin vermilion males.

Because of difficulty of separation, I have placed the cherry and the eosin females in one general class. For the same reason I have placed the eosin and the cherry vermilion males in one general class.

eosin ♀ by cherry verm. ♂ = { eosin ♂ 119; cherry ♀ 154 }
{ eosin ♀ ....; cherry ♀ .... } 110
{ eosin ♂ ....; cherry ♂ ....20; cherry verm. ♂ ..... } 76
eosin verm. ♂ 18

The results may be accounted for in the following way:

P eosin.............. ♀ XcVe — XcVe
cherry verm........ ♂ XcvE — ——

F_1 FEMALE. F_1 MALE.
XcVe XcVe
XcvE ——
cherry ♀ eosin ♂

GAMETES OF F_1 ♀ XcVe — XcvE — Xcve — XcVE
GAMETES OF F_1 ♂ XcVe — ——

F_2 FEMALES.		F_2 MALES.	
XcVe XcVe	eosin ♀	XcVe ——	eosin ♂
XcvE XcVe	cherry ♀	XcvE ——	cherry verm. ♂
Xcve XcVe	eosin ♀	Xcve ——	eosin verm. ♂
XcVE XcVe	cherry ♀	XcVE ——	cherry ♂

In concluding I wish to express thanks to Dr. Morgan for furnishing me with material with which to carry on these crosses and for his helpful suggestions during the progress of the work. I have also been greatly helped by discussion of the theoretical side of the matter with Messrs. A. H. Sturtevant, H. J. Muller and C. B. Bridges. Mr. Bridges' suggestions and his final reading and criticism of the manuscript were directly responsible for the appearance of the paper in its present form. I must not forget to express my appreciation of the assistance of my friend Benjamin Schwartz.

PARTHENOGENETIC CLEAVAGE OF THE ARMADILLO OVUM.

H. H. NEWMAN.

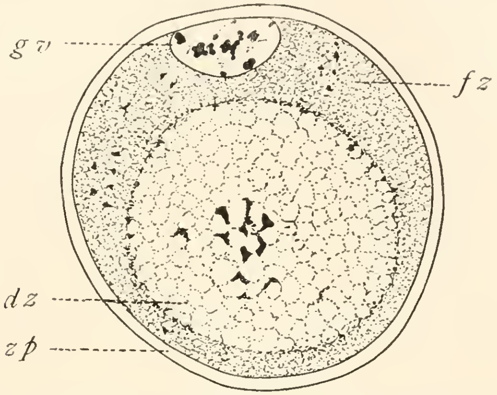
INTRODUCTION.

In the year 1909 I began a general study of the ovary of the nine-banded armadillo in search of some explanation of the phenomenon of polyembryony. Among the first of the peculiar features to catch my attention were what appeared to be cleavage stages in certain types of ovarian ova. It was thought that this condition might have some bearing on polyembryony but further study and a review of the literature on kindred topics convinced me that these changes were in no sense to be interpreted as stages in normal development. They must, on the contrary, be interpreted as more or less abnormal and abortive attempts, on the part of ovocytes that have reached maturity but have been denied the normal culmination of their destiny in ovulation and fertilization, to develop embryos in spite of insuperable difficulties. I should hesitate to contribute another chapter to the already voluminous literature on the supposed parthenogenesis of mammalian ova during follicular atresia had I not at my command evidence of a very crucial character, which seems to me to demonstrate beyond controversy a certain amount of real parthenogenesis in a mammal.

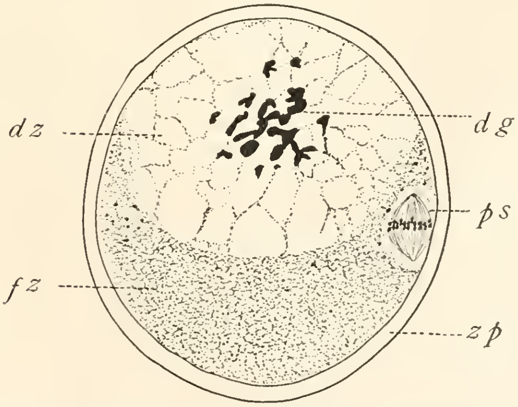
The ovaries studied are the same as those that formed the basis of a recent paper on maturation and fertilization in this species (Newman, 1912). Those ovaries, fixed in freshly made Zenker's fluid and stained by Bensley's copper chrome hæmatoxylin process, give the clearest pictures.

RÉSUMÉ OF THE EVENTS OF MATURATION LEADING UP TO PARTHENOGENETIC CLEAVAGE.

In order that the changes preceding cleavage may be understood it will be necessary to review the facts dealing with the maturation process which were described in a recent paper (Newman, 1912). The most striking peculiarities of this process have to do with the reorganization of the cytoplasmic materials of the ovocyte. In this respect, and probably in many others, the armadillo bears a marked resemblance to the marsupials, especially *Dasyurus*, the Australian native cat, whose early development is so ably described by Hill (1910). So close is this resemblance that any description given for one species would apply in almost every detail to the other.



TEXT-FIG. 1. A full-grown ovocyte, showing cytoplasmic organization, etc. Deutoplasmic zone (*dz*), formative zone (*fz*), germinal vesicle (*gv*), zona pelucida (*zp*) (× 410).



TEXT-FIG. 2. A maturing ovocyte, showing the new reversed polarity. The ovocyte is placed with the animal pole upwards. The deutoplasmic zone (*dz*) occupies the animal pole, the formative zone (*fz*) occupies the vegetative pole. The polar spindle (*ps*) lies in a tangential position at the equator of the ovocyte. Deutoplasmic granules (*dg*) lie in the center of the deutoplasmic mass. The zona pelucida (*zp*) is a dense envelope, without radiations.

The full grown ovocyte of the armadillo has the structure shown in text-figure 1. The cytoplasm shows two clearly defined regions, a peripheral zone of deeply staining homogeneous protoplasm, the *formative zone* (*f.z.*), and a central lightly staining, spherical mass of coarsely reticular or alveolar material, the deutoplasmic zone (*d.z.*), in which occur irregular masses of coarse and deeply staining granules (*d.g.*), the supposed equivalent of the yolk or deutoplasmic granules of the ova of marsupials. The germinal vesicle (*g.v.*) is flattened against the dense zona pelucida (*z.p.*) at the animal pole and in it the chromatin is seen as tetrads or in other forms characteristic of the prophases of maturation. Such an ovum has the appearance of being centrolecithal. Coincident with the formation of the first maturation spindle a radical rearrangement of the two cytoplasmic zones occurs. The deutoplasmic sphere pushes its way to the surface at the animal pole and crowds the formative material to the vegetative pole where it assumes the form of a cap, thick at the pole and thinning out to a feather edge at the equator (see text-figure 2). The polar spindle, in a stable metaphase (*p.s.*), occupies a position as near the animal pole as possible without leaving the formative material and has its axis tangential to the periphery of the ovocyte though parallel with that of the main axis of the cell. This spindle is evidently insulated from the surrounding formative protoplasm by a capsule of non-staining material, and has no astral rays.

A very large number of ovocytes were found in exactly this condition and it seems certain that the progressive changes normally come to a standstill at this point. The next step in normal development is ovulation, the stimulus of which initiates the completion of the maturation process. Such resting ovocytes occur in large follicles whose granulosa cells are intact and whose follicular fluid is homogeneous, abundant, and stains deeply with hæmatoxylin and allied stains. In any normal ovary during the period of œstus there are many ovocytes of this sort, all in practically the same condition and equally ready for ovulation. Probably such mechanical factors as size of follicle or nearness to the periphery determine which follicle shall rupture first. Fertilization of this one egg seals the fate of all the remaining ovocytes of the same generation in that they can never go through the process of ovulation and therefore cannot develop normally. A very small percentage of these ovocytes continue the maturation process to the extent of completing the first maturation division; a still smaller proportion (only three out of hundreds of cases examined) complete the second maturation; while the remainder, over 90 per cent., are struck by the processes of follicular atresia and either go into early cytolysis or enter upon a period of parthenogenesis. It is with the latter contingency that the present paper deals.

Abstriction of the Deutoplasmic Material and Its Subsequent Fate.

The condition of equilibrium, just described and illustrated in text-figure 2, is normally disturbed only by ovulation, but, in the case of ovocytes whose normal history has been cut short by the fertilization of an ovum and the formation of a corpus luteum, the equilibrium may also be upset by the marked changes in the chemical environment incident to follicular atresia. The follicle in which atresia has set in rapidly decreases in size through the loss of follicular fluid, but, even before the amount of fluid has materially diminished, its chemical composition is clearly altered, for it no longer stains deeply with the ordinary cytological stains but remains pale gray under hæmatoxylin treatment. Just what the nature of the change is I am unable to determine, but there can be no question as to its radical character. As atresia continues, the lumen of the follicle gradually disappears, partly through the loss of fluid and partly through the ingress of cells from the disintegrating granulosa layer of the follicle. With this radical alteration of chemical environment the ovocytes show marked changes, whose significance it is our problem to determine.

The first act of the ovocyte entering upon parthenogenetic development is the abstriction of the deutoplasmic mass from the formative protoplasm. That this is a practically universal phenomenon can scarcely be doubted for there are in the present material literally hundreds of instances of it, and one can with confidence look for some stage of the process in every follicle that has reached the mid-period of atresia. Abstriction of the deutoplasm is essentially an act of rejuvenation on the part of a dying cell, in that the still living protoplasm is freed from the burden of the inert by-products of metabolism. The exact mechanics involved in this purification process can scarcely be determined from the fixed material, but appearances seem to warrant the conjecture that the very fluid deutoplasmic material escapes from the cell membrane by the rupture of the latter and that, with the subsequent closure of the ruptured membrane, the deutoplasm becomes strictly extra-cellular and without effect, except in a secondary mechanical way, upon the living protoplasm of the germ cell. Freed from its burden the renewed egg-cell rounds up into an approximately spherical form and apparently floats in the deutoplasmic fluid. It is of considerable interest to note that a similar abstriction of deutoplasmic material occurs as the initial step of normal development in *Dasyurus.* Hill shows that this discarded material plays no further part in development but merely occupies any available space within the persistent zona pelucida that is not occupied by the blastomeres. Finally it comes to lie in the cleavage cavity of the blastula and is probably gradually absorbed. In view of the striking similarity in the early history of the deutoplasm in the two species it is difficult to avoid the conclusion that the process, as described for these ovarian ova of the armadillo during atresia, is a close approximation of the first step in normal development, about which we know practically nothing at present.

The deutoplasmic fluid, laden with its masses of deutoplasmic granules, usually gives the appearance of a multicellular body, but I am inclined to consider this condition as largely an artefact. It is not improbable that the strong fixing fluids used coagulate this somewhat viscous material in the form of many small rounded masses. In a few cases, as in Figs. 2 and 4, the deutoplasm is only slightly broken, but in the majority of instances we find advanced fragmentation, as in Figs. 1, 3, 5, etc. Whether the deutoplasm occurs in one or many fragments the deutoplasmic granules occupy a central position in the various pieces giving each fragment the illusory appearance of a nucleated cell. These are not however to be interpreted as in any sense true cells. At best they may be designated as *cytoids*. Other writers who have studied similar conditions in mammals have referred to the existence of multicellular conditions in which some cells possess nuclei and others do not. I suspect that, were the truth known, these fragments without nuclei would turn out to be of deutoplasmic origin. Very commonly these cytoids in our material form a pseudo-epithelial layer surrounding the true cells, as in Figs. 3 and 5, or less distinctly in Fig. 1. In other cases they are distributed at random among the cellular products of cleavage (Figs. 7–11) and serve only to give a false appearance of cellular multiplicity and to confuse and obscure the pictures of cleavage.

The Reëstablishment of Normal Interrelations Between the Nucleus and the Cytoplasm and the Resultant Formation of Cleavage Spindles.

The egg, much reduced in size but with a renovated cytoplasm, is now the seat of a renewal of nucleo-cytoplasmic exchanges, as evidenced by the appearance of an extensive system of astral rays at the two poles of the spindle. It will be recalled that the nucleus during the period prior to ovulation was a naked spindle, enclosed in an insulating capsule and entirely devoid of astral radiations. Such a spindle is evidently an isolated system and lies inert in the cytoplasm. The renovated cytoplasm of the egg from which the deutoplasm has been extruded now bears a different chemical relation to the nucleus and the change is shown in the disappearance of the capsule about the nucleus and in the appearance of typical astral radiations. That a renewal of metabolic relations between nucleus and cytoplasm is one of the most essential facts of normal fertilization has been recently maintained by Lillie (1911) on the basis of his studies of fertilization in *Nereis*, and there is reason to believe that this is the physiological explanation of parthenogenesis wherever the latter is found, whether normal or experimental. In this case it is my conviction that the sudden change in the chemical character of the cytoplasm restores the basis of life and growth to the egg and that cleavage is the natural consequence. In such cases as that shown in Fig. 1 the nucleus has returned to a resting state and has every appearance of undergoing healthy changes which should culminate in cell division by mitosis. It is impossible to state positively that such a nucleus has regressed from the spindle condition shown in text-figure 2, but that such is the case seems at present to be a reasonable conjecture. In eggs of this sort there is a characteristic zone of activity in the cytoplasm at a short distance from the nuclear membrane which may indicate active interchanges between nucleus and cytoplasm. The chromatin is in the form of a series of beaded threads resembling a spireme and therefore may be interpreted as in a very early prophase of the first cleavage. Soon the chromosomes begin to condense and the typical appearances of later prophases pass over into unmistakable mitotic figures like that shown in Fig. 4, which can be interpreted in no other way than as cleavage spindles. The so-called cleavage mitoses of other writers on this subject have been interpreted by opponents as belated maturation spindles which have been displaced and distorted by the abnormal conditions of atresia; but such an interpretation of the figures here described would seem very far-fetched in view of the fact that these spindles are so radically different from the naked, insulated spindles of maturation. I have never seen a vestige of astral radiations in a polar spindle, while the radiations in a number of such cases as that shown in Fig. 4 are as obvious and as extensive as those which I have observed in the cleaving eggs of annelids and other favorable material, and far clearer than similar appearances in fish eggs or other less favorable material. The spindles are frequently abnormal in form, being not uncommonly tri- or multipolar; and sometimes the poles are ill defined, as in Fig. 4, but the radiations are always very distinct. When the figure is multipolar the chromatin distribution is very irregular and nests of nuclei (like those shown in Fig. 12) are produced without any division of the cytoplasm. It is very common to find such multinucleate but unicellular eggs and I am inclined to attribute their existence to such irregular mitoses as that just mentioned. Certain considerations lead me to venture a conjecture as to the mode of origin of these multipolar spindles. It is not uncommon to find in ovocytes of the type shown in text-figure 2 various steps in the anaphases of the first maturation division, which might readily result in the formation of two nuclei without the extrusion of a polar-body. Conditions like those shown in Figs. 2 and 3 are not nearly so abundant as those like Fig. 1, but they are about as common as are multipolar spindles and might readily result in the latter type of abnormality. Eggs with such paired nuclei have the general appearance of fertilization stages but cannot be interpreted as such, unless the reunion of the polar nucleus with its sister, the nucleus of the secondary ovocyte, could be considered a sort of fertilization process. It is not difficult, however, to see how double nuclei of this sort could form just such multipolar spindles as that shown in Figs. 13 and 14. It will be readily noted from the figures that these abnormal spindles have a very much larger number of chromosomes than sixteen, the number seen in all clear maturation figures. This in itself militates against the interpretation of these phenomena as maturation processes, and is in accord with the conjecture that the figures may be the result of the coöperation of two nuclei derived from a maturation division.

The bipolar spindles, such as that shown in Fig. 4 (see also Figs. 15 and 16), also contain more than the haploid number of chromosomes and cannot on that account be maturation figures.

Early Cleavage Stages Resulting in the Formation of a Few Blastomeres.

In the course of these studies several very pretty two-cell stages have been encountered of which those shown in Figs. 5 and 6 are typical. The stage shown in Fig. 5 is a perfect two-cell stage in which both nuclei are in the resting phase. There are no other nucleated bodies within the zona pelucida, but a pseudo-epithelium of deutoplasmic cytoids forms a capsule about the two blastomeres. A clear case of a two-cell stage in which the second cleavage is well under way is shown in Fig. 6. The cleavage spindles are cut transversely in both cells, one of which shows an early and the other a later anaphase.

After the second cleavage the subsequent divisions are less normal and the picture of blastomeric regularity is confused. The spindles lose distinctness, as a rule, and the chromatin begins to break down, giving appearances like those shown in Figs. 7 and 8, which are two sections through the same egg. Another example of the same condition is shown in Figs. 9 and 10, also taken from one egg. In Fig. 7 can be seen one cell with a fragmenting nucleus, a degenerating nucleus in another cell, and an incomplete mitotic figure with only a few small chromosomes. In Fig. 8 occur three cells all of which show abnormal, though unmistakable, mitotic figures. Such an egg might be considered as a six-cell stage, with a prospect of reaching a ten-cell condition. There is every evidence, however, of approaching death and disintegration in such cases and one would not be inclined to look for much further developmental progress in such unpromising material. In the egg shown in Figs. 9 and 10 there are several imperfect spindles and two small but healthy nuclei. One might be somewhat more optimistic about the ultimate fate of cases of this sort, but, since these cases show about the maximum of development in the present material, such optimism is scarcely warranted.

Fig. 11 is introduced especially to show an unusually fine mitotic figure in one of several cells in an egg in which three other cells show more or less normal nuclei. This egg occurred in a follicle in a very advanced stage of atresia and it is rather surprising to find so pronounced a spark of life in a structure so nearly dead.

In all of the cases cited and in nearly all in which similar phenomena were observed the zona pelucida was dense and quite intact, hence there can have been no invasions of stroma or follicular cells. So all nuclei found must be products of the division of the original germinal vesicle. In more advanced atresia, however, the zona begins to open up cracks which admit hordes of stroma cells and leucocytes that feed upon the disintegrating egg material and form cell masses that have been interpreted by some authors as the products of embryonic development. In such cases the observer might be inclined to interpret such synthetic structures, which frequently have an epithelial structure, as tissues, equivalent to those formed in normal embryonic development. Leo Loeb ('11 and '12) in recent papers interprets as placental tissues certain structures found under similar conditions in the guinea-pig ovary. Not having seen his preparations, but simply judging by his microphotographic illustrations, I am inclined to think that, since he gets no intermediate or advanced cleavage stages, the cell complexes he discusses are open to the interpretation just given for similar phenomena in the armadillo, about whose synthetic origin there appears to be no doubt. Parthenogenetic development in all probability goes no farther in the armadillo than the stages illustrated in the figures. That true parthenogenesis begins and proceeds for a few steps seems assured, but it seems highly improbable, *a priori*, that development could long continue in an environment so unfavorable as that afforded by follicles in which atresia has made such progress as we have seen.

Discussion of the Literature on Parthenogenesis in Mammals.

In 1900 Bonnet in an able paper entitled "Giebt es bei Wirbeltieren Parthenogenesis?" reviewed all the literature dealing with parthenogenesis in vertebrates and gave much space to the question of parthenogenesis in mammals. He concluded that there was no incontrovertible evidence of this mode of development in any of the contributions dealing with the changes described as occurring in ovarian ova during follicular atresia. A considerable number of authors had described ovarian ova containing centrally situated mitotic figures which they had interpreted variously as cleavage spindles or as belated maturation figures. Multicellular masses within zona pelucida were described, in which some cells contained nuclei and others were without nuclei. Some writers considered this condition as a result of a degenerative fragmentation of nucleus and cytoplasm, and others were convinced that the cell mass was the product of parthenogenetic cleavage. Another class of writers called attention to the existence of various kinds of more or less complex ovarian dermoids, epithelioma and other teratoma, which by some were interpreted as the end product of the parthenogenetic development of ovarian ova. Judicially examining all of the evidence before him Bonnet concludes that all of the mitotic figures seen in ovarian ova are to be considered not as cleavage mitoses but as more or less abnormal maturation figures, that the so-called multicellular embryos are degenerative products, and that teratoma and other kindred phenomena must be explained in some other way than as the products of parthenogenetic cleavage of ovarian ova. This conclusion is, I believe, fully justified by the evidence then available.

Since this review and judgment of Bonnet several authors have reopened the question but have failed to agree. Spuler (1900) insists that the figures which he finds located in the center of ovarian ovocytes, after the extrusion of one polar body, are cleavage spindles, because normally ovulation occurs before this stage and the ovocytes do not develop thus far unless fertilized. He places especial emphasis on finding one ovum in which a centrally located spindle occurred in an egg that had two polar bodies. This, however, is probably a case similar to that described by the present writer (Newman, '12) as due to a precocious division of the first polar body, the spindle being merely the second maturation spindle. Spuler fails to add any material strength to the affirmative side of the question reviewed by Bonnet.

Van der Strict (1901) working with the bat ovary also took the affirmative side of the question as the result of his discovery that ovocytes of the second order occasionally divided mitotically into two cells of approximately equal size. This division could not, he thought, be considered as an anomalous polar body formation, nor as a mitotic division due to degeneration, nor as fragmentation, but only as the beginning of parthenogenetic division. I am inclined to think that Van der Strict was dealing with phenomena closely akin to those described in the present paper for the armadillo, but he failed to make his evidence especially convincing.

L. Loeb (1901) takes a position similar to that assumed by Spuler and Van der Strict and goes a step further in that he holds that the subsequent fragmentation of the egg material into nucleated and enucleated pieces is a progressive phenomenon akin to the parthenogenetic development of the egg of *Chætopterus* which, as Lillie has shown, develops into a larva of considerable complexity without any nuclear cleavage. Loeb figures one multicellular mass in which a single cell is dividing mitotically, but the spindle is of the size and character of a polar spindle and lacks astral rays. Under the circumstances such a spindle would probably be capable of interpretation as the first polar spindle of an egg that had undergone degenerative fragmentation.

Rubaschkin (1906), as the result of his studies of the guinea-pig ovary, takes a decidedly negative stand, maintaining that all mitotic figures occurring in ovarian ova during follicular atresia are more or less distorted or otherwise abnormal maturation mitoses, and in no sense the mitoses of embryonic cleavage. Multinuclear and apparent multicellular conditions are interpreted by him as the result of degenerative changes brought about by the conditions incident to follicular atresia.

Athias (1908 and 1909) once more reviews the whole situation and presents further data derived from the study of a number of species of mammals. After summing up the evidence pro and con he finds himself unable to reach a definite conclusion though leaning decidedly to the negative point of view. Pending an extensive program of investigation on the subject he prefers to reserve judgment.

My own investigations herewith presented were begun, as stated, in 1909 and, as the reader will have discovered, support the view that a limited amount of parthenogenetic cleavage occurs but that development proceeds no farther than two or three cell divisions.

SUMMARY.

The evidence derived from a study of large numbers of armadillo ovaries demonstrates, I believe, that parthenogenetic cleavage takes place in atretic follicles of this species of mammal. Whether cleavage is preceded by maturation is not clear, but I am inclined to believe that no polar bodies are extruded in those ovocytes that are destined to undergo cleavage.

There is strong reason to believe that the abstriction of the deutoplasmic material in these ovarian ova is equivalent to one of the steps in normal development, for a similar process occurs in the development of several marsupials, the ovogenesis of which shows the same history of deutoplasm formation and reorganization as that seen in the armadillo.

There is no evidence that cleavage proceeds beyond the eight-cell stage, and even at that period there are many signs of advancing degenerative processes. It seems certain, therefore, that, in the armadillo at least, no complex tissue masses such as ovarian teratoma or epitheliomata result from a continuation of the process of parthenogenetic cleavage.

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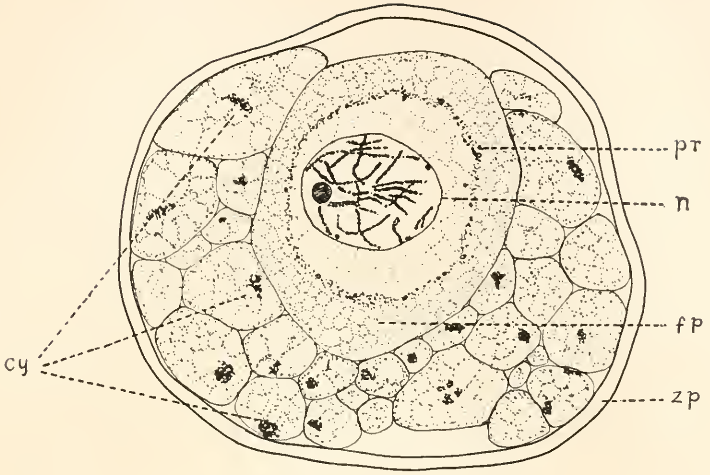
Hull Zoölogical Laboratory,
University of Chicago,
April 8, 1913.

EXPLANATION OF PLATES.

PLATE I.

FIG. 1. Egg with large resting nucleus, showing resumption of chemical interaction between nucleus and cytoplasm preparatory to the formation of the first cleavage spindle. The deutoplasm has been extruded and has been fixed in the form of numerous cytoids (*cy*) that almost surround the egg. The dense zona peludica (*z.p.*) encloses both egg and cytoids. The egg shows a zone of unmodified formative protoplasm (*f.p.*) and a precipitation ring (*p.r.*), where the changes between nucleus and cytoplasm are most active. Note that each cytoid has a centrally located, deeply staining, mass of granules, which are the deutoplasmic granules of earlier stages and are not to be interpreted as degenerating nuclei. (× 800.)

FIG. 2. Egg in which the deutoplasm has just been abstricted and has not yet undergone fragmentation to any marked extent. Two nuclei (n^1 and n^2) lie side by side giving the appearance of a fertilization stage. It is probable that these are the product of the first maturation division, completed without the extrusion of a polar body. This could readily occur in cases where the maturation spindle occupies a central position. These two nuclei would doubtless form a multipolar spindle like that shown in Figs. 13 and 14 and would probably result in the formation of nests of nuclei, as in Fig. 12. Other labelling as in Fig. 1. (× 800.)



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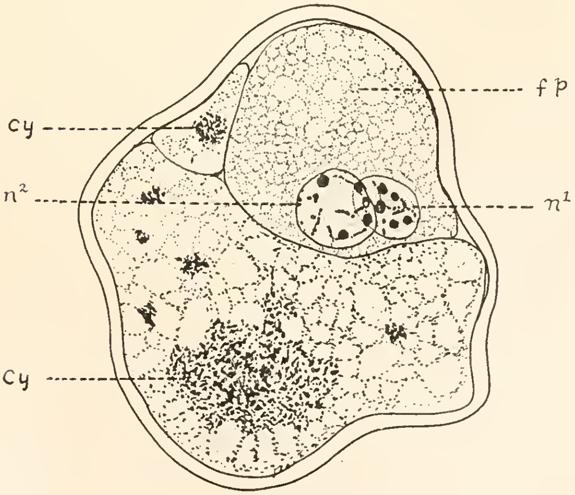
fp
cy
n²
n¹
cy

2

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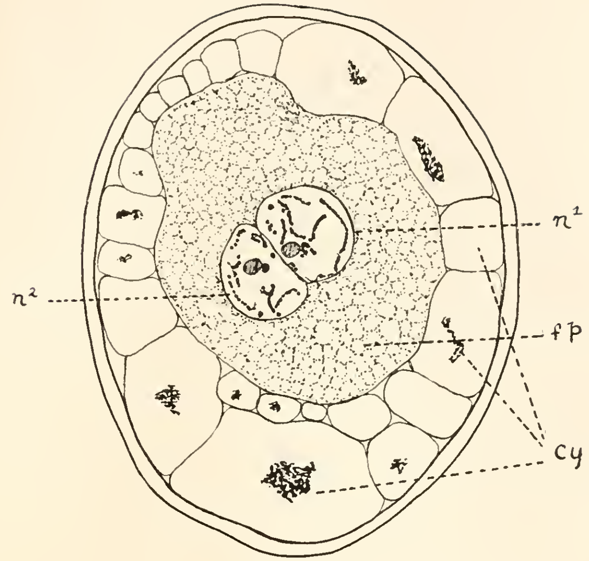
PLATE II.

FIG. 3. Egg with pseudo-epithelium of cytoids and with binucleated egg cell in the center. The explanations given for Figs. 1 and 2 will apply here. (× 800.)

FIG. 4. Egg showing mitotic spindle (*m.s.*) with well-defined polar radiations running from nucleus throughout the cytoplasm. The rather blunt-ended spindle shows mitosis in an anaphase and there are many more than the reduced number of chromosomes, which one invariably finds in maturation spindles. Such a spindle cannot be other than a cleavage spindle. There are only two large cytoids (cy^1 and cy^2). (× 800.)



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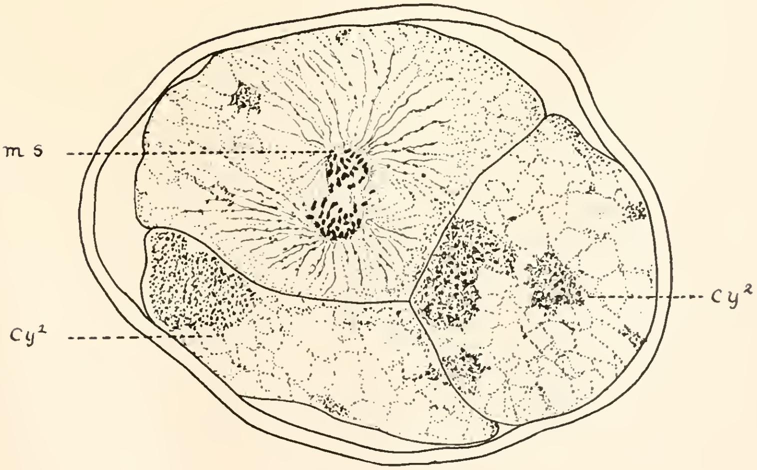

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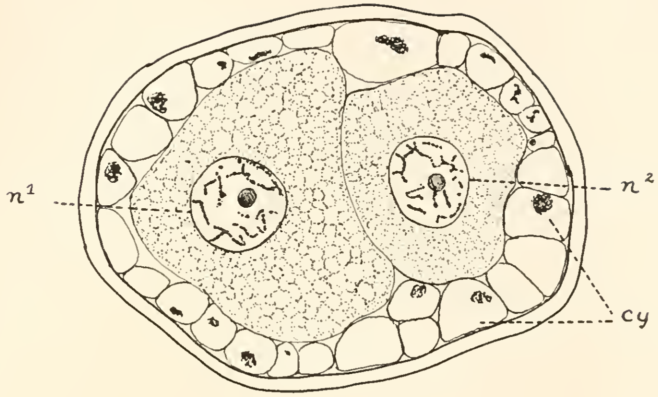
PLATE III.

FIG. 5. A two-cell stage of cleavage. The two cells are of somewhat unequal size, but the nuclei (n^1 and n^2) are both in good condition and in the resting stage. A pseudo-epithelium of deutoplasmic cytoids surrounds the blastomeres. (× 800.)

FIG. 6. A two-cell stage passing into the four-cell condition. Two mitotic spindles are cut transversely to the axis. The spindle on the right ($m.s^2$) is cut near the equatorial plate and shows only a few of the chromosomes. Other chromosomes are scattered through several sections. The spindle on the left ($m.s^1$) is in a late anaphase and has two groups of chromosomes, one at each end of the spindle. The present section cuts through only one of these chromosome groups. Large numbers of cytoids occur in other sections. (× 800.)



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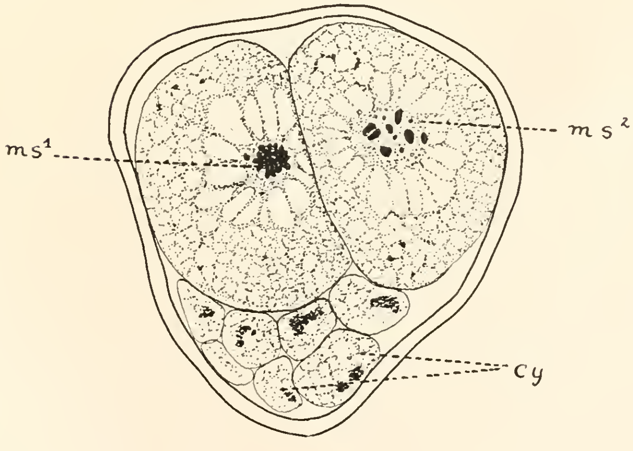

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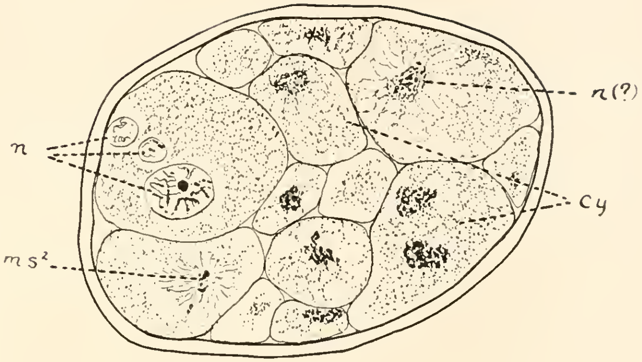
PLATE IV.

FIGS. 7 and 8. Two sections through the same egg, showing one clearly defined mitotic spindle (*m.s*^{1}), a cell containing three nuclei in the resting stage, one cell with what appears to be a degenerating nucleus (*n*?), and one cell, shown in both sections, with an imperfect mitotic spindle and only a few chromosomes (*m.s*^{2}). Cytoids of all sizes are mixed in with the blastomeres. Such an egg seems to be in about a four-cell stage, but will probably not go much further. (× 800.)



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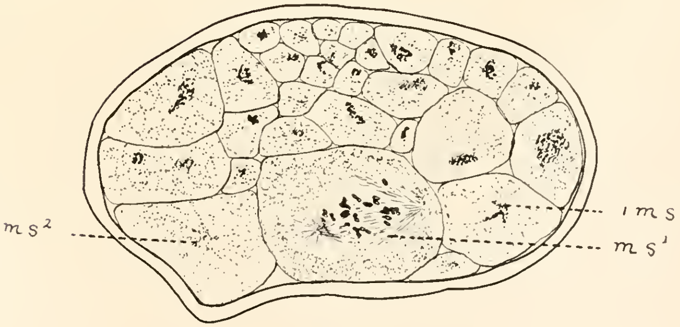

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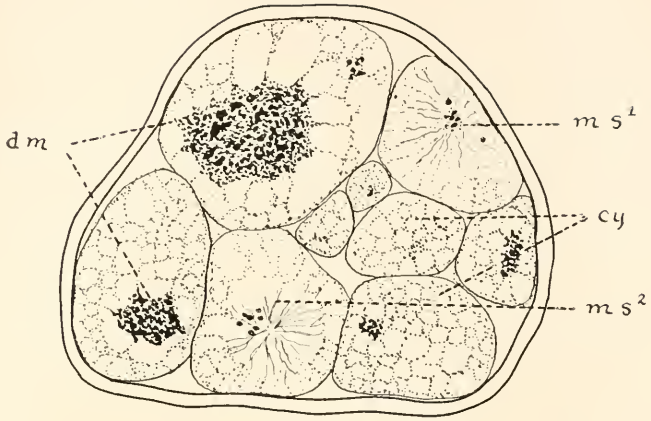
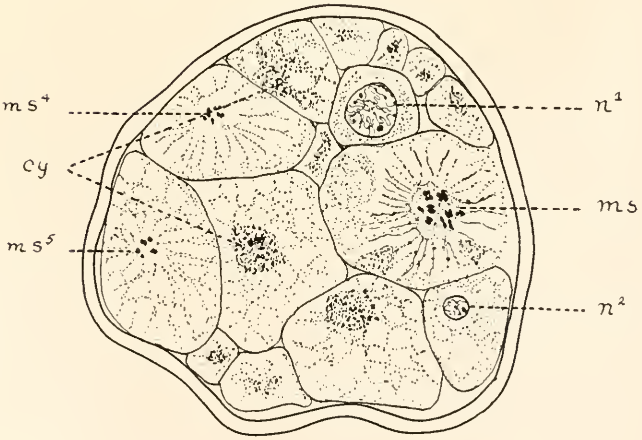
PLATE V.

FIGS. 9 and 10. Two sections through one egg. This seems to represent about an eight-cell stage. There are two cells with resting nuclei (n^1 and n^2) and three cells with more or less perfect mitotic spindles ($m.s^1$–$m.s^5$). Cytoids are scattered among the blastomeres, giving the appearance of cell multiplicity. (× 800.)


dm
ms¹
cy
ms²

9


ms⁴
cy
ms⁵
n¹
ms
n²

10

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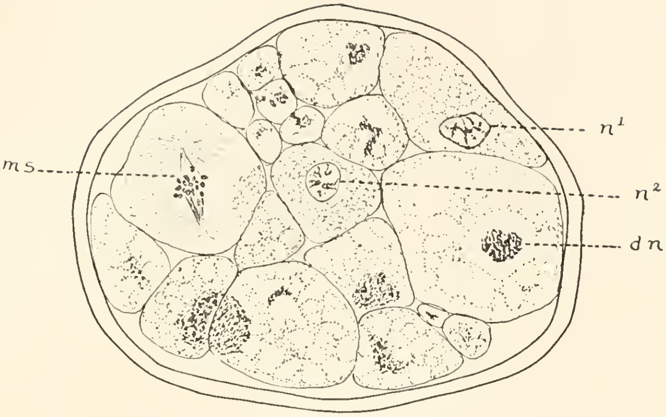
PLATE VI.

FIG. 11. A section through an egg in about a four-cell condition showing the most normal cleavage spindle (*m.s*) found in all the present material. Two other cells show unmistakable resting nuclei (n^1 and n^2). (× 800.)

FIG. 12. An egg with many small nuclei in the formative protoplasm. Such a condition may have arisen through the formation of a multipolar spindle like that shown in Figs. 13 and 14. This is a very frequently found condition. (× 800).



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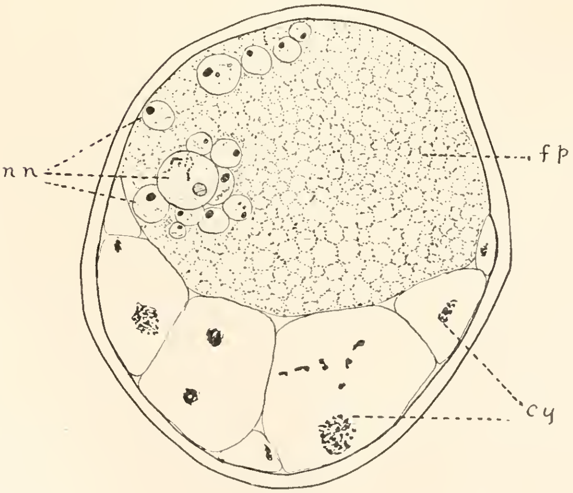

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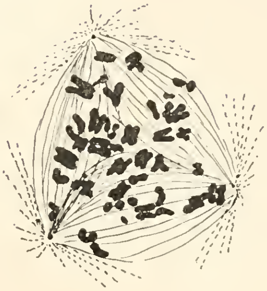
PLATE VII.

FIGS. 13 and 14. Shows the entire chromosome complex in a multipolar spindle which may have arisen from the fusion of two nuclei in eggs like those shown in Figs. 2 and 3. It will readily be seen that the number of chromosomes is very much higher than would arise from a spindle formed from a single nucleus. (× 1,600.)

FIG. 15. An equatorial plate view of a first cleavage spindle of the bipolar type. The number of chromosomes is far larger than the haploid number characteristic of maturation figures. (× 1,600.)

FIG. 16. Another example of the same phenomenon shown in Fig. 15. (×1,600.)



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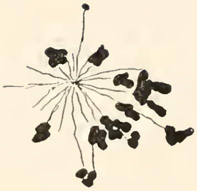

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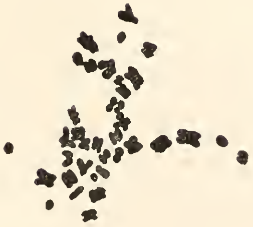

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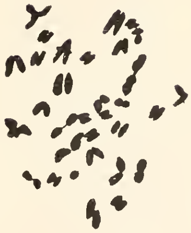

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BIOLOGICAL BULLETIN

THE REACTIONS OF CERTAIN ANIMALS TO GRADIENTS OF EVAPORATING POWER OF AIR. A STUDY IN EXPERIMENTAL ECOLOGY.

VICTOR E. SHELFORD.

WITH A METHOD OF ESTABLISHING EVAPORATION GRADIENTS BY
V. E. SHELFORD AND E. O. DEERE.

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I. INTRODUCTION.

Aside from studies of the hygienic workers (Rubner and others), studies of mountain sickness, and other physiological phenomena of high altitudes and reduced atmospheric pressure (Cohnheim and others), little attention has been given to the effect of loss of water upon land animals. Since evaporation is determined by air movement, humidity, pressure, temperature, and indirectly by illumination, most of the so-called physical factors are measured in combination by instruments measuring evaporation.

A knowledge of the effect of evaporation upon animals is important for the following reasons: (1) Because knowledge of the relationships of land animals to the surrounding medium is important from the standpoint of evolution and physiology, (2) because factors controlling distribution are effective in proportion to their effect upon the organisms concerned, and (3) because animals kept under laboratory conditions in experiments in behavior, genetics, etc., are often subjected to constantly changing atmospheric conditions and these changes may be sufficiently important to interfere with the results and the validity of the conclusions drawn. With these points in mind, the writer and Mr. E. O. Deere undertook to construct a piece of apparatus for the control of atmospheric conditions, but especially to establish experimental gradients and to test the reactions of animals to variations in the rate of evaporation. Various difficulties were experienced in getting the apparatus into working order and it was necessary for Mr. Deere to leave when the apparatus was ready for the control of humidity and some half dozen experiments had been performed. Nearly all of the experiments accordingly devolved upon the writer and we present the method of work only, as a joint contribution.

II. A Method of Establishing Evaporation Gradients.

By Victor E. Shelford and E. O. Deere.

The air supply was obtained by a compression pump as shown in Fig. 1. A metal funnel (*MF*) covered with cheese cloth conducted the air to a pipe, 14 cm. in diameter and 340 cm. long, to a Beach-Russ Vacuum pump, no. 1, run by a one-half horse-power motor. The air left the pump through a ¾-in. iron pipe, and entered a ½-in. Crane oil separator, which removed the oil with which the pump is operated. In the pipe near the oil separator there was a pressure gauge, an air-cock opening to the exterior, and an automatic blow-off set for 5 lb. pressure. From here the air passed through 15 m. of ¾-in. galvanized iron pipe, the central 5 m. of which was surrounded by a 1½-in. iron pipe water-jacket, connected with the city water supply by means of a ¼-in. iron pipe, provided with a valve. By turning the city water supply into this jacket we were able to lower the temperature of the air when the outside air was warmer than the room. This was necessary because after the flows were divided they passed varying distances before reaching the tanks and if the temperature of the air was higher than that of the room, different amounts of cooling gave different temperatures in the experimental boxes. Several brass ground unions made it possible to take the entire line apart. The air current was divided into six parts by means of iron pipe Y bends, and reduced to ¼-in. with bushings. Each branch was provided with a

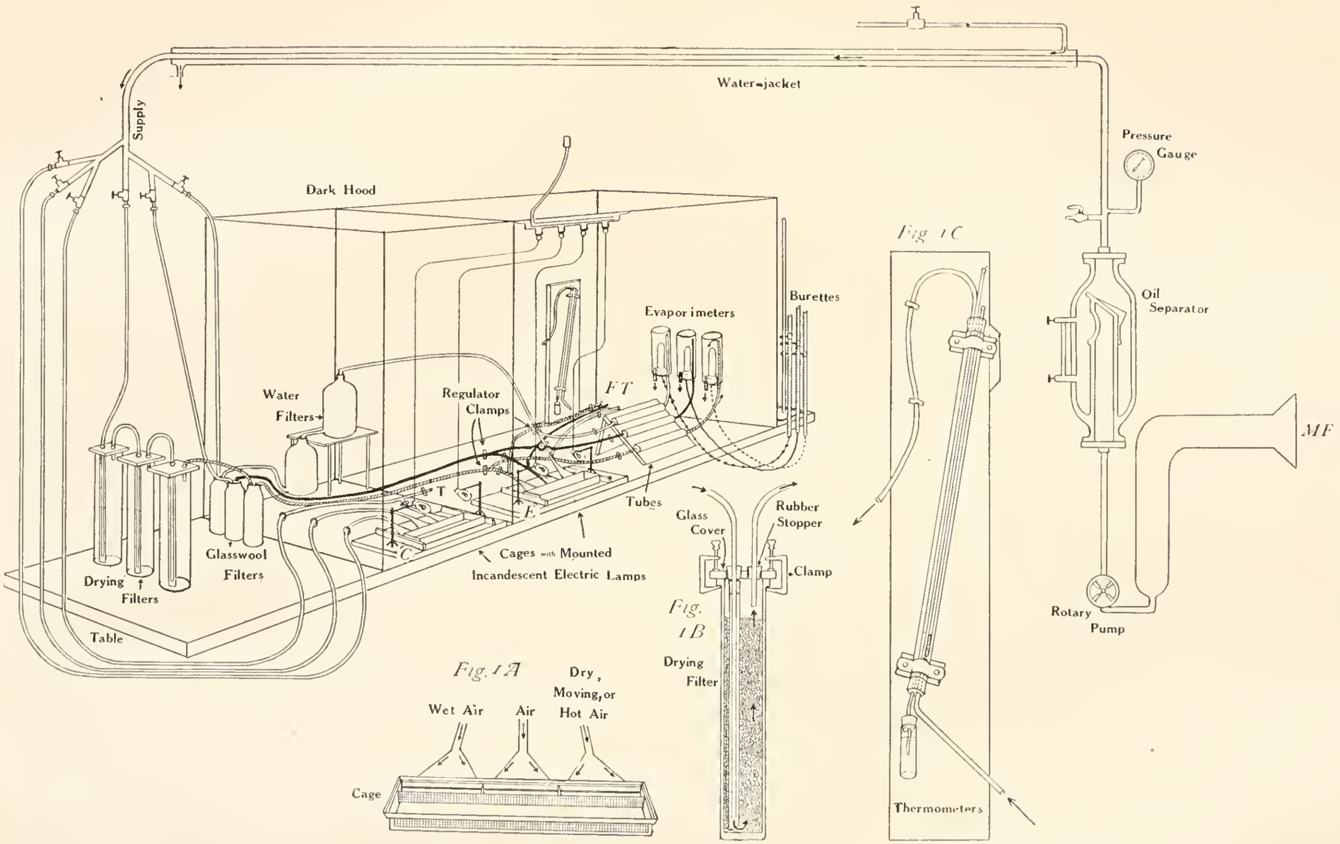
Water-jacket
Supply
Pressure Gauge
Oil Separator
MF
Rotary Pump
Dark Hood
Burettes
Evapor imeters
FT
Water Filters
Regulator Clamps
T
E
C
Tubes
Cages with Mounted Incandescent Electric Lamps
Glasswool Filters
Drying Filters
Table
Fig. 1C
Thermometers
Glass Cover
Rubber Stopper
Clamp
Fig. 1B
Drying Filter
Fig. 1A
Wet Air
Air
Dry, Moving, or Hot Air
Cage

FIG. 1. Showing the apparatus for the control of the evaporating power with enlarged drawings of an experimental cage (Fig. 1*A*), — a drying filter (Fig. 1*B*), and the wet and dry bulb thermometers (Fig. 1*C*). *MF* is the metal funnel intake; *T*, a tube connected directly with the pump which may be connected with any of the tubes marked *FT*, directly or with a heating coil interposed. *C* is the control cage; *E*, the experimental cage. The latter is supplied with air from the various filters. For further description see text.

rising-stem straight-way valve, with hose end nipple attached by means of a $\frac{1}{4}$-in. brass ground joint union.

The experimental boxes (Fig. 1*A*) for the gradient experiments were designed by the senior author and were 6.5 cm. wide by 30 cm. long, and 2.5 cm. deep. The animals were confined in a portion of each box, 5 cm. wide, by a screen (8 meshes to the centimeter). Air was supplied to the boxes by means of three fish-tail burner-shaped introducers, opening into a narrow slit along the rear midway between top and bottom. The area of the slit in each introducer was approximately the same as the area of the 7 mm. brass tube which connected it with the pump. The air after leaving the fishtail burner-shaped introducer passed through the screen that confined the animals away from the slit, across the box, and out of the front through a screen similar to the first. The air tended to spread out at about the angle of the side of the introducer, and a small piece of metal was inserted between the rear wall and the confining screen, to deflect this part of the flow directly across the box. Each experimental box rested upon a movable board to each end of which a ring stand was fixed. Each ring stand bore a universal clamp, a small piece of Bessemer rod, and a single burette clamp used to hold a 1 candle-power incandescent lamp in any desired position. Our experiments were performed with the center of the incandescent filaments 20 cm. from the bottoms of the experimental boxes, and one fourth of the distance from each end. These boxes were placed in a hood painted dead black, and provided with two curtains, one of which hung from above downward and contained a small slit for the observer, the other when in position came up from below to a point 10 cm. above the level of the lamp. This excluded practically all the faint light of the room, and the light was between the animals and the observer which made it less easy for them to see him.

The control box (*C*) was supplied either with untreated air direct from the pump or with no air. The experimental box (*E*) and Fig. 1*A*, when experiments with atmospheric humidity were being performed, was supplied with three kinds of air, wet at one end, dry at the other, and untreated or medium air in the center. The dry air was rendered dry by passage through three or more sulphuric acid filters, Fig. 1*B* (see Shackell, '09). Each filter consisted of a Whitehall Tatum museum jar, 45 cm. tall and 9 cm. in diameter at the top. The covers were pieces of plate glass, 13 mm. thick and provided with two holes 25 mm. in diameter. They were clamped into place by ordinary hardware screw-clamps with a 6 cm. opening. The contact points were filed smooth and covered with rubber tubing. The air entered through a glass tube inserted in a rubber stopper in one of the two holes in the cover just mentioned. In order to conduct air to the bottom of the jar, the tube through which it entered was inserted into a second cork fitting inside a larger tube (Fig. 1*B*) so that when the glass plate was pushed into place, this stopper was pushed inside the glass tube so as to make an air-tight connection. The air left the filters from the top through a short tube inserted in the second rubber stopper. The filter jars were filled with crushed pumice stone, the pieces ranging from 0.5 mm. to 7 mm. in diameter, as indicated by the meshes of the screens used in separating and removing the finest and coarsest pieces. This was impregnated with crude sulphuric acid.

After leaving the drying filters, the air passed through glass wool filters, made by filling ordinary 1-liter, wide-mouthed wash bottles with glass wool. Tests of the air, made by bubbling the supply from these filters through a methyl-orange solution for a period of 30 minutes, indicated that no sulphuric acid passed into the final delivery pipe.

The untreated air entered the final delivery pipe after passing through a glass wool filter. The wet air passed from the glass wool filter through two 2-liter aspirator bottles filled with crushed pumice impregnated with distilled water. To test the evaporating power of the three kinds of air, Livingston ('06, '08, '10*a*, '10*b*, '11; see Abbe, '08) cup *atmometers* (evaporimeters) were used. Small Whitehall Tatum museum jars, similar to those used for the sulphuric acid filters, 19 cm. deep inside, and 9 cm. in diameter at the neck, were used to confine the atmometers. The atmometers were placed in position, after being stoppered with one-holed rubber stoppers with small tubes inserted. A large stopper with three holes was used to carry all the apparatus inside the jar. The tubes which connected them with burettes, where the amount of water evaporated was read off, passed through the central hole of the large stopper. Another tube which connected with the air supply passed through one of the side holes, parallel with the evaporimeter and reached within about 5 mm. of the bottom of the jar. The apices of the evaporimeter cups were from 25–30 mm. from the bottom. The third hole in the stopper was used for the exit pipe; the air from the supply passed upward to the bottom of the jar, which was inverted, and in returning to the exit tube, passed over the atmometer. The size of the jar was such that the velocity of the air over the atmometers was the same for a given flow, as across the cages. An atmometer thus enclosed was provided for each of the three kinds of air. For testing relative humidity (Fig. 1*C*), two long chemical thermometers, graduated to 0.1 of a degree, were inserted inside of a glass tube 50 cm. long and 22 mm. in diameter. One of the thermometers was provided with a wick of absorbent cotton toweling, which dipped into a vial of water. Air from a supply pipe could be introduced into the tube below the bulbs.

Two glass Y's were inserted between the filters and the experimental box, in each air line. The respective stems of these connected with the main air tube and with the experimental cage, as indicated in Fig. 1. One arm of the Y of each line was connected permanently with one arm of the Y attached to the experimental box and supplied with a pinch-cock. The six free Y arms were supplied with free rubber tubes as shown in Fig. 1. The three of these connected with the nearest filters could be joined to the observations tubes (Fig. 1), or to the atmometers, or the thermometer tube. The other three (*FT*) could be used to connect the experimental cages directly with *T* of the control tube to give a rapid flow or when a coil of aluminum pipe submerged in hot water was interposed, to give warm air. These six free tubes made possible cross connections and the shifting of any kind of air to any section of the experimental cage. All open tubes were closed by means of pinch-cocks.

For studying details of behavior and testing the ability of the animals to withstand high rates of evaporation, three glass tubes, each with a total length of 21 cm. and an inside diameter of 32 mm., were used. These were connected with the free rubber tubing of the less frequently used arms of the Y's by means of funnels of the same diameter as the inside of the tubes. The stems of these were inserted into single-holed rubber stoppers. The large end of the funnel was covered with a screen whose meshes were 1 mm. square, and the whole inserted inside the tube, with the stem projecting outside the rubber stopper for the attachment of the rubber tube. The funnel permitted the expansion of the air so as to practically fill the tube when it entered, and the screen prevented the small insects from entering the rubber hose. The air left each tube through the small funnel, similarly inserted into the other end. Since animals find difficulty in walking on curved glass surfaces, the lower third of each tube was filled with paraffin upon which sand was sifted while the paraffin was still warm. From each of the tubes the air passed to an atmometer chamber, as previously described, so that the evaporation could be recorded while the animals were being observed.

In order to vary dryness only, it was necessary that the flows of the three kinds of air be the same. The flows were measured by collecting the air for a period of five seconds in a jar filled and inverted in a vessel of water. The pump commonly delivered air under sufficient pressure to force it through the filters and give a flow of from 12 to 16 liters per minute, an amount sufficient to change the air in the part of the cage immediately in front of each introducer (when all are flowing, in the entire cage) in a maximum of .6 of a second.

It was possible to adjust the flows so that they were essentially alike, without collecting the gas. The boundaries of the rear walls of the cages were of solid metal and the slit was midway between top and bottom. A small triangle of thin paper 2 to 3 cm. long and about 1 cm. across the base was taken between the thumb and fore-finger by the point. With the hand held firmly by resting against the cage, the paper was placed in a position such that it was entirely in contact with the upper half of the rear wall without bending. The piece of paper was then lowered so that the broad end came in front of the slit and the deflection of the paper was noted and the flows adjusted until each gave the same deflection. The maximum flow was barely sufficient for effective experiments.

The atmometers were those furnished by the *Plant World*. Three were selected with the standard .75 and restandardized by the careful adjusting of the flows until they were exactly alike. The evaporation was recorded for standard lengths of time when the flows of the medium and dry air were turned alternately over each of the atmometers for several periods of ten minutes and one period of an hour. It was found that the evaporation of one kind of air was the same no matter which evaporimeter was used. At the end of the experiments, the atmometer used with the wet air showed smaller evaporation due in part to sand that was accidentally blown out of the observation tube onto the atmometer.

III. MATERIAL.

The following species were studied: the yellow-margined millipede (*Fontaria corrugate* Wood), ground beetles (two species of *Pterostichus*), the wood frog (*Rana sylvatica* LeC.), the red-backed salamander (*Plethodon cinereus* Green), the sticky salamander (*Plethodon glutinosus* Green) and several species of snails, all from moist forest situations, maximum evaporation is about 11.5 c.c. per day near the surface of the ground; the common toad (*Bufo lentiginosus* Shaw), the digger wasp (*Microbembex monodonta* Say), the bronze tiger beetle (*Cicindela scutellaris lecontei* Hald.), the spiders (*Geolycosa wrightii* Em. and *pikei* Marx), all from dry sand ridges covered with cottonwoods and pines, a type of situation where the maximum evaporation per day is about 32.5 c.c. The animals were kept in the laboratory under as nearly natural conditions as possible. With the exception of *Plethodon* and *Fontaria*, they were kept only a few days.

IV. Experimental Results.

1. *Dry Air.*

The air used in the experiments was dried in the sulphuric acid filters described on page 82. The water vapor present after treatment depended upon the humidity of the original air, upon the temperature, the rate of flow, and the number of filters used. On account of variations in temperature and relative humidity from day to day, it is necessary to either practically saturate all the air with water vapor or to dry all of it and follow by standard treatment at a constant temperature if the same conditions are to be produced from day to day. Tables I. and II. (pp. 88, 96) show from 0.5 to 1.05 c.c. evaporation for 20-minute periods (calculated from 10-minute exposures) and relative humidity of 9 to 20 per cent. for the treated air. The relative humidity of the air used ranged from 40 to 60 per cent. of saturation; the reduction in per cent. of humidity ranged from 34 to 52. The moist air was more constant and the evaporation is arbitrarily given as 0.02 c.c. per 20 minutes. This number is based upon a number of one-hour exposures of the atmometers, as readable results were not noted in 10-minute exposures.

(*a*) *Moist Forest Animals.*

1. *Physiological Effect and Reactions.*—With the exception of the snails the dry air was stimulating to all the animals tried. *Plethodon cinereus* was stimulated at once in the driest air and usually moved back and forth in the observation tubes. Activity sometimes alternated with short periods of coiling but movement was the rule and was usually increased during the first fifteen minutes when erratic movements occurred. These were usually followed by coiling or cessation of activity accompanying a dry appearance of the skin. The animals usually dried and shriveled without further activity, the ability to move being gradually reduced. In the medium air, when the rate of evaporation was low, they often behaved quite normally for a few minutes when

coilings and activity began to alternate. This was followed by heightened activity and stiffening as before. In the gradient a negative reaction to air of more than a minimum evaporating power was clearly shown as indicated by Table I., and Chart I., Experiment 11 (p. 87). The salamanders appeared to sense the drier air at once as indicated by hesitation or by turning back when the change was encountered. The latter indicates that these animals have a sense of orientation in the gradient. They usually tried the driest air one or more times and the different trials were usually followed by turning when it was encountered again. They usually piled together in the moist air after 13 to 18 minutes and remained so for considerable periods. *P. glutinosus* is clearly more sensitive to dryness than is *P. cinereus*. While the former was clearly more stimulated in both observation tubes and gradient experiments than was the latter, stiffening due to drying appeared so early that in the gradient experiments *glutinosus* did not turn back as definitely as did *cinereus*.

The wood frog was stimulated at once in the dry air; it showed agitation at first but very soon (a few seconds to five minutes) crouched close to the bottom, drew the legs close to the body, and partially or wholly withdrew and closed the eyes. After this had continued for a time, the frog usually hopped in the direction in which it was headed and if the disturbance was not relieved it repeated the crouching.

While the frogs sometimes appeared to orient in the gradient, this capacity is poorly developed and the graphs are quite different from those of the salamanders. The frogs showed a preference for the moist air and avoided the dry air mainly by random hops and a lesser tendency to hop in the moist air. The difference in the appearance of the animals in the different parts of the experimental cages was striking. In the air of highest and medium evaporating powers the withdrawal and closing of the eyes just referred to took place to a degree apparently proportional to the rate of evaporation. Here the skin was dry and dull. In the moist air, the difference after a few moments exposure, was striking. The skin glistened with moisture, the frog sat upright, the eyes were fully protruded and wide open, and the animals gave an impression of sagacity not

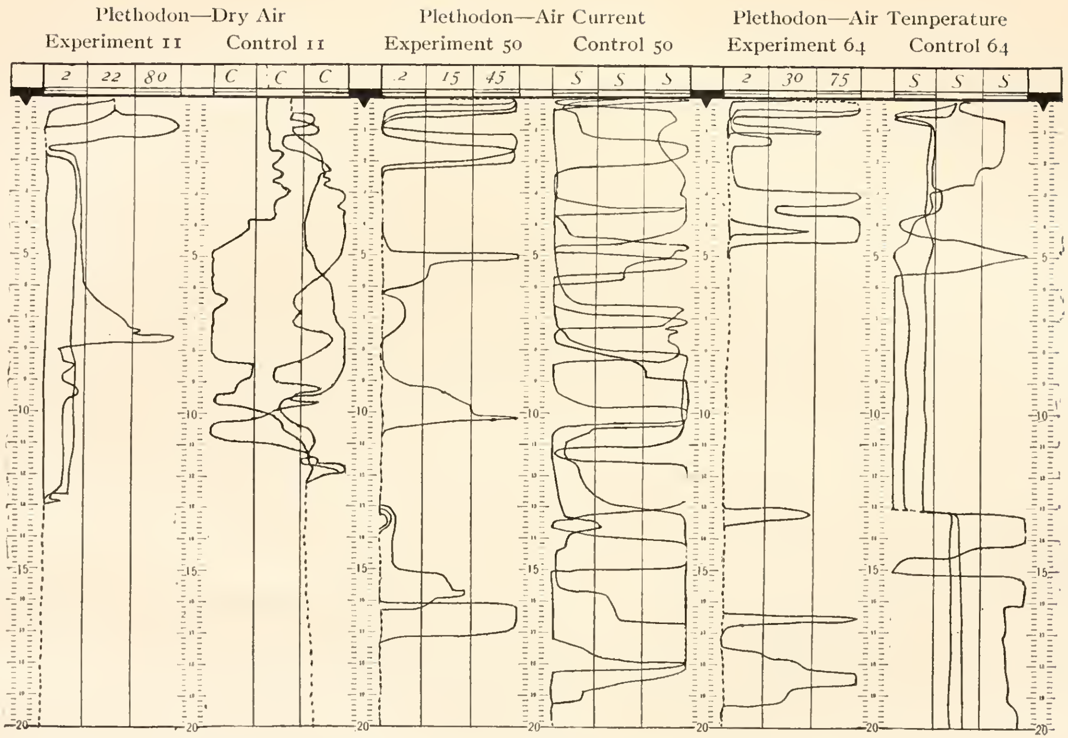

CHART I. Showing similarity of reaction of *Plethodon cinereus* to high evaporation due to dryness, current and higher temperature. Distance from right to left between the scales represents the length of the cage. The vertical scales are time scales with minutes divided into ten second periods. The tracings represent the movements of the individual animals; horizontal distance represents distance lengthwise of the cages and vertical distance represents time. The solid vertical lines separate the divisions corresponding to the thirds of the cages. The numbers at the heads of these columns in the experiments represent the evaporation in hundredths of cubic centimeters during the 20 min. experimental periods. In the controls the letter *C* indicates that a current was flowing while *S* indicates standing air. The similarity of the negative reaction to the highest evaporation is clear in the three cases; the avoidance of it was least in the experiments where the evaporation was least rapid. The control of Expt. 11 shows the typical bunching of the individuals in conditions of considerable evaporation.

TABLE I.

Showing the reactions of moist forest animals to gradients of evaporating power produced by different amounts of atmospheric moisture. Flow 12 to 16 liters per min. Three individuals were used. The experiments bearing a number and the letter A were performed with the individuals used in the experiments bearing the same number without the A:—*e. g.*, the control individuals of 5 were used in expt 5A, etc. The experiments lasted 20 min. except where B and C appear. These experiments lasted 40 min. or an hour; 9B is the 2d, 20 min., 9C the third. Averages of the controls are averages of all controls including those shown in tables IV and VI. Experiments starred were performed with E. O. Deere. Length; *Rana* 2 to 3 cm.; *Plethodon* 5 to 8 cm.

No. of Experiment.	Species.	Experiment.												Control.					
		Evaporation in c.c. During Experiment.			Relative Humidity in Per Cent. of Saturation.			Turned Back From		Per Cent. of Time in Thirds.			Temperature of Experiment.	Turned Back From		Per Cent. of Time in Thirds.			Temperature of Control.
		Moist.	Medium.	Dry.	Moist.	Medium.	Dry.	Moister.	Dryer.	Moist.	Medium.	Dry.		Corresponding to Moister.	Corresponding to Dryer.	Corresponding to Moist.	Corresponding to Medium.	Corresponding to Dry.	
5*	*P. cinereus*............	0.02	0.40	0.80	—	—	—	2	5	82	14	4	26	2	0	33	40	27	26
5A*		0.02	0.40	0.80	—	—	—	8	14	63	28	9	26	4	3	26	53	21	26
5X	(duplicate control).													7	7	11	12	77	
11		0.02	0.22	0.80	98	56	20	1	5	93	5	2	22	4	3	61	34	5	22
57		0.02	0.40	0.90	98	61	9	0	2	75	21	4	21	1	1	36	16	48	21
4*		0.02	0.25	0.60	98	54	16	0	8	86	9	5	21	4	3	38	13	49	21
Average:		0.02	0.33	0.78	98	57	15	2	7	80	15	5	23	2.8	2.5	36	28	36	23
58	*P. glutinosus*...........	0.02	0.30	0.80	98	60	10	0	7	91	7	2	22	0	1	50	48	2	21
60	*Rana*.................	0.02	0.30	0.60	—	—	—	0	0	33	45	22	22	0	0	36	39	25	22
7*		0.03	0.30	0.75	96	54	16	0	1	72	26	2	22	1	0	29	23	48	22
10		0.03	0.30	0.90	96	56	19	0	0	82	18	0	24	1	1	27	26	47	22
59		0.02	0.45	0.50	—	—	—	0	0	65	20	15	22	2	2	43	51	6	22
6*		0.02	0.25	0.50	98	54	16	1	2	74	12	14	21	2	2	51	18	31	
6A*		0.02	0.25	0.50	98	54	16	2	2	41	28	31	21	1	1	36	30	34	21
Average:		0.02	0.31	0.74	97	55	17	0.4	2.4	66	22	12	22	1	1	36	30	34	22

Table I.—*Continued.*

No. of Experiment.	Species.	Experiment.											Control.						
		Evaporation in c.c. During Experiment.			Relative Humidity in Per Cent. of Saturation.			Turned Back From		Per Cent. of Time in Thirds			Temperature of Experiment.	Turned Back From		Per Cent. of Time in Thirds.			Temperature of Control.
		Moist.	Medium.	Dry.	Moist.	Medium.	Dry.	Moister.	Dryer.	Moist.	Medium.	Dry.		Corresponding to Moister.	Corresponding to Dryer.	Corresponding to Moist.	Corresponding to Medium.	Corresponding to Dry.	
9	*Fontaria*	0.02	0.30	0.90	98	56	19	1	2	47	21	32	24	0	0	39	17	44	24
9B		0.01	0.30	0.90	98	56	19	1	2	67	16	17	24	0	1	46	21	33	24
9C		0.01	0.20	0.60	98	56	19	1	1	55	16	29	24	1	1	45	23	32	24
12		0.02	—	0.50	—	—	—	1	2	62	25	13	20	2	1	38	26	36	20
56		0.02	0.45	0.90	98	61	9	0	3	48	22	30	21	4	1	46	19	35	21
62		0.02	0.45	0.60	98	46	12	2	3	54	17	29	24	2	1	21	16	63	20
Average:		0.02	0.34	0.73	98	55	15	1	2.3	56	19	25	24	1.2	.9	39	21	40	22
14	Carabid	0.02	—	0.75	—	—	—	2	7	80	15	5	20	1	1	56	7	37	20

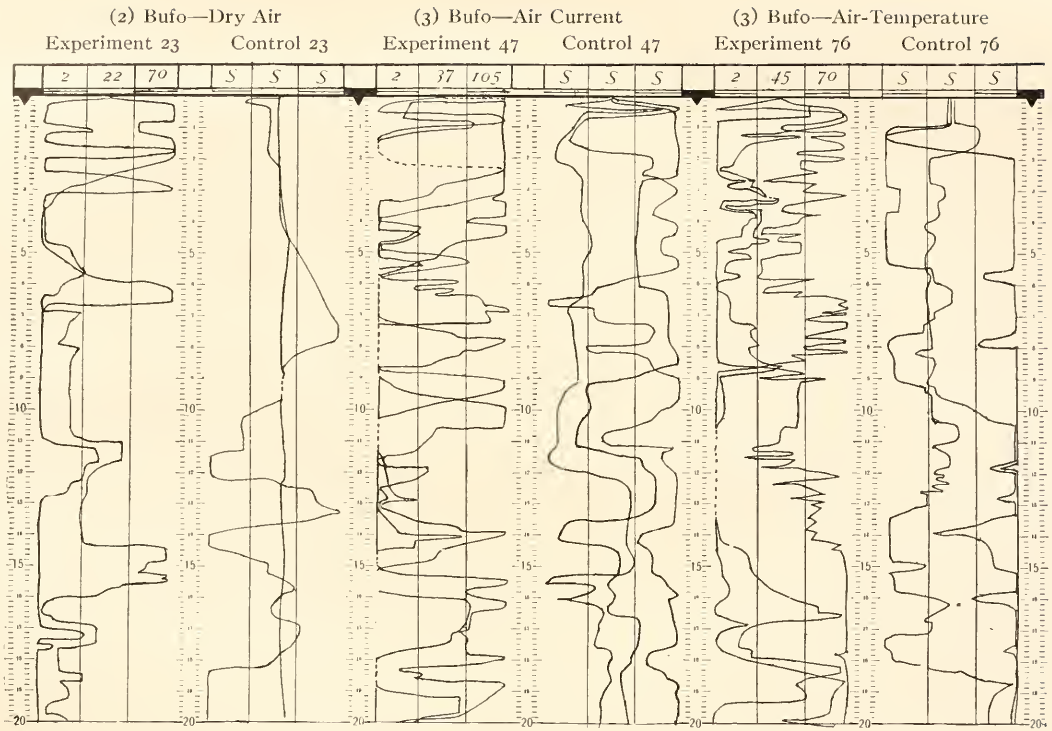

Chart II. Shows the negative reaction of *Bufo* to air of high evaporating power. The avoidance of the dry and moving air is clear. The reaction to the hot air is marked by peculiar short back and forth movements and the avoidance was a little less sharp than in the other cases. Comparison of this chart with Chart I. (p. 87) gives a good idea of the relative avoidance of the same or similar conditions by two amphibians that usually spend the day under cover and come out on cloudy days and at night. The difference is associated with habitat preference.

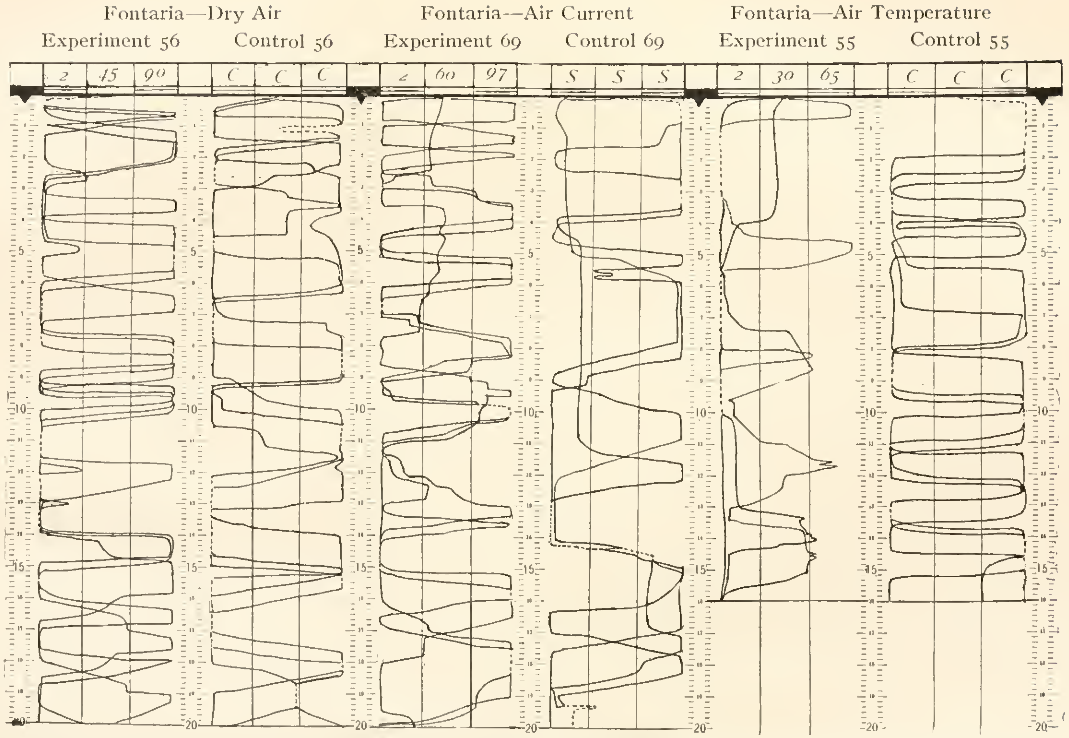

CHART III. Showing similarity of reaction to current and dryness and a sharper reaction to temperature in experiments with *Fontaria*. The small number of turnings from the dry and moving air is evident; turnings are replaced by hesitation at the boundary of the highest evaporation rate and short stays in the dry and moving air. The avoidance of the higher temperature is sharp.

belonging to the anurans. In a few experiments with *Chrophilus nigritus*, it was found that this frog possesses a similar set of reflexes.

The ground beetles (*Pterostichus adoxus* (1) and *pennsylvanicus* (2) were very sensitive to the dry air, *P. pennsylvanicus* particularly so (Expt. 14, Chart V., p. 97). Here two specimens of *pennsylvanicus* tried the dry air a few times and then began to hesitate and turn back. The individual of *adoxus* was less active but after one trial of the driest air and one turning from the medium, came to rest in the moist air as did the *pennsylvanicus* after a number of trials and turnings and at the end of fifteen minutes. The loss of the stock of *pennsylvanicus* necessitated the repetition of the experiment with *adoxus* alone. In the second trial, the beetles when put in the center dashed lengthwise of the cage once and by chance all bunched together in the driest air, due to thigmotaxis and gregarious tendencies. Soon they became very much stimulated and single individuals dashed to the moist end of the cage and back with great speed. This bunching interfered with the reaction and at the end of the 20-minute period only two had worked out a preference for the moist air.

Fontaria was clearly stimulated by the dry air after less than five minutes exposure in the observation tubes. The individuals in the medium air showed less activity than those in the driest. Individuals in the moist air remained quiet most of the time. In most cases in the gradients (Chart III., Expt. 56, p. 91) the *Fontarias* entered the driest air a number of times and then began to show greater activity in the dry end, to stay a shorter time there, to hesitate upon entering, and to turn back occasionally. Their reaction to the dry air was clearly negative as shown by a time preference for the moistest air, and the haltings and turnings even, though the ability to orient in the gradient seems poorly developed.

Snails and slugs are not good for gradient experiments of short duration because of their sluggishness. The slug (*Philomycus carolinensis* Bosc.) was usually inactive in the moist air but quite active during the first half hour in the dry air. Here the tentacles were withdrawn in less than five minutes and remained so until death ensued. Inactivity or very slow movements were characteristic of the second, third and fourth half-hour periods. Marked reduction in size was evident in about two hours. They died after two and one half hours.

Comparable results were obtained with several snails. *Polygyra thyroides* Say, when active at the time put into the observation tubes, behaved as follows. In the moist air activity continued intermittently. The animals retreated into the shell from time to time and usually remained stuck to the side of the tube for half an hour or more. In the dry air withdrawal into the shell followed in five minutes but partial extension sometimes continued from time to time during the first half hour. In one individual, a fresh epiphragm was formed at the end of two hours and shrinkage and withdrawal into the shell continued during several hours of observation. *Polygyra palliata* Say behaved similarly. Active individuals put into dry air became inactive but more quickly than *thyroides*. When put into the tubes in an inactive state and with strong epiphragms no activity occurred in either medium or dry air. In the moist air the foot was protruded after 45 to 55 minutes and creeping began after 70 minutes.

On one occasion specimens of several species were taken from the same stock jar and placed in the moist air together, with the following results: *Polygyra palliata* Say and *P. thyroides* Say became active in 10 to 20 minutes, *Pyramidula alternata* Say in 80 minutes, *Polygyra fraudulenta* Pit. showed no activity at the end of three hours and forty minutes but was found moving 14 hours later, a night having intervened. The experiments were carried far enough to show that activity may ordinarily be induced in faint light by air nearly saturated with moisture but it is clear that other factors are concerned because occasionally it is not induced and when so, frequently does not continue.

(*b*) *Sand Dune Animals.*

Of the sand area animals studied, the common toad is least characteristic of sandy situations because toads belonging to the same species are found in moist woods, and because toads of the dunes breed in the pools and not on the dunes.

Furthermore these toads are probably physiologically different from toads of moister situations. The toads are the only sand animals used that clearly avoided dry air. The stimulation was less marked than that of the wood frogs and was not accompanied by striking or characteristic reflexes. Activity was greater in the controls of gradient experiments where uniform current was used than where still air was used (Chart II., Expt. 23, p. 90). In the gradient the toads were negative to drier air (Table III.) but turned back much less definitely than did the salamanders. Rapid random movements appeared to be characteristic. The ability to orient in the gradient is poorly developed.

The spiders (*Geolycosa*) appeared not to be affected by the moist air. When observed in the tubes, no differences between the individuals in the different conditions could be noted. In the gradient experiments (Table II.) a positive reaction to dry air was clearly shown when the spiders were induced to move about. It was necessary to select individuals of the same sex and of about the same size, as these animals manifested a very striking repulsion for one another and when one spider came near to another one or both darted away with great speed. Thus when one spider moved, three being present, more movement usually resulted and if none of the spiders was killed in combat the experiment resulted successfully. In many cases however, especially when differences in size or sex occurred, some of the spiders usually were killed before the experiment ended. Experiment 29, Chart IV., shows a typical graph characterized by the erratic dashes made by two individuals when meeting.

The digger wasps (*Microbembex*) were likewise slightly positive (Table II.) to dry air, though their chief reaction was digging (Chart V., p. 97). The digging reaction took place in the medium and moist air but not in the dry. There was no special activity in the dry air. In Chart V. the reaction of three individuals in a gradient is shown; the crosses indicate that the wasp was digging at the half minute opposite which the cross appears. It will be noted that the crosses are all in the moist and medium sections.

A few experiments were tried with grasshoppers from sand

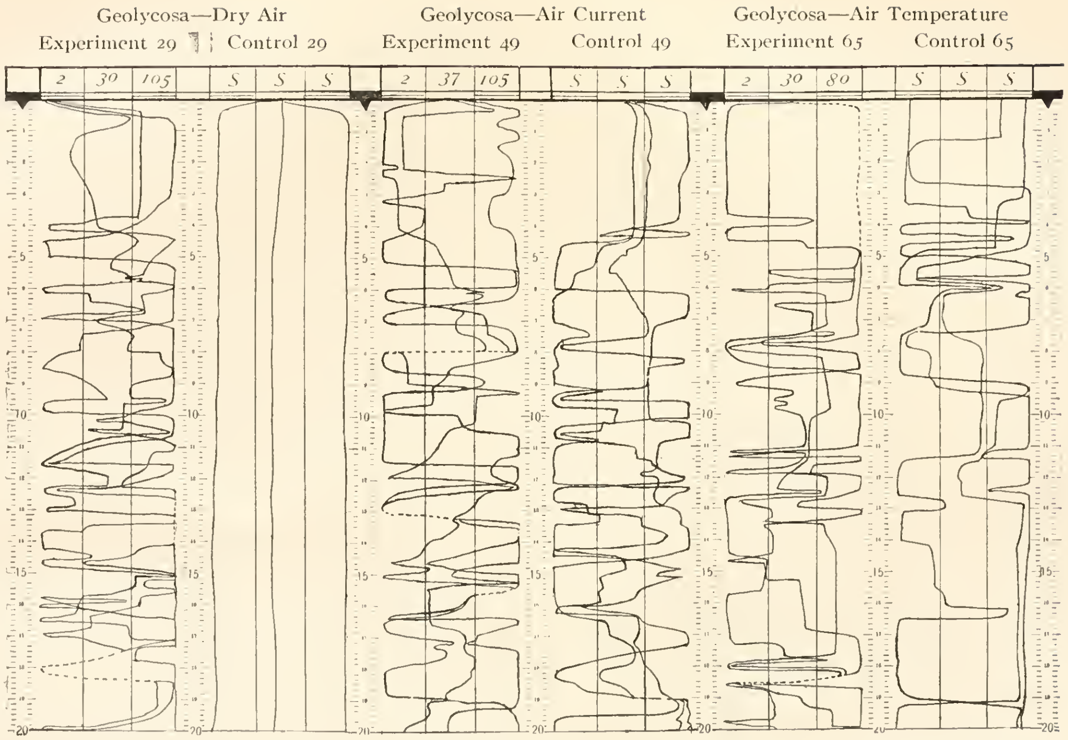

CHART IV. Showing the reactions of *Geolycosa* to air of high evaporating power. An avoidance of the *lowest rate* of evaporation is clear in all cases. A comparison with Chart III. (p. 91) shows the behavior of two Arthropods of roughly comparable habits but different habitat preferences.

TABLE II.

Showing the reactions of cottonwood dune animals to gradients of atmospheric humidity; 20-minute experiments. Flows 12–16 liters per minute. See description of Table I. Averages of the controls are the averages of all the controls including those in Tables IV. and VI. Length: *Bufo*, 2.5 cm.

No. of Experiment.	Species.	Experiment.												Control.					
		Evaporation in c.c. During Experiment.			Relative Humidity in Per Cent. of Saturation.			Turned Back From		Per Cent. of Time in Thirds.			Temperature of Experiment.	Turned Back From		Per Cent. of Time in Thirds.			Temperature of Control.
		Moist.	Medium.	Dry.	Moist.	Medium.	Dry.	Moister.	Dryer.	Moist.	Medium.	Dry.		Corresponding to Moister.	Corresponding to Dryer.	Corresponding to Moister.	Corresponding to Medium.	Corresponding to Dryer.	
23	*Bufo*..................	0.02	0.22	0.67	—	—	—	1	9	66	22	12	20	2	2	12	76	12	20
42		0.02	0.22	0.75	—	—	—	2	6	55	16	29	23	5	9	37	22	41	23
43		0.02	0.22	0.75	—	—	—	11	19	62	31	7	23	3	2	49	32	19	23
61		0.02	0.45	0.90	98	40	10	11	21	50	35	15	24	5	3	37	15	48	24
Average:		0.02	0.31	0.76	98	40	10	6	14	58	26	16	22.5	8.0	8.1	28	34	38	22.5
28	*Geolycosa*..............	0.02	0.45	0.75	—	—	—	2	0	24	17	59	20						
29		0.02	0.30	1.05	—	—	—	11	17	28	13	59	20	0	0	33	34	33	20
30		0.02	0.30	0.90	—	—	—	0	2	63	28	9	20	0	0	31	3	66	20
32		0.02	0.22	0.81	—	—	—	1	0	58	10	32	19	0	0	7	48	45	19
Average:		0.02	0.32	0.68	—	—	—	3.5	2	43	17	40	20	1.8	1.8	29	29	42	20
22	*Microbembex*...........	0.02	0.22	0.67	—	—	—	12	10	23	62	15	20	9	12	49	21	30	
24		0.02	0.30	0.52	—	—	—	14	8	12	46	42	21	14	19	35	32	33	20
26		0.02	0.30	0.60	—	—	—	18	17	30	49	21	21	6	10	9	73	18	21
27		0.02	0.22	0.81	—	—	—	21	16	30	49	21	20	7	5	29	25	46	20
33		0.02	0.22	0.79	—	—	—	5	6	18	54	28	19	9	4	19	37	44	19
34		0.02	0.22	0.81	—	—	—	18	14	32	34	34	19	12	11	33	33	34	19
Average:								14.6	12	24	50	26	20	7.1	7.6	29	37	34	20

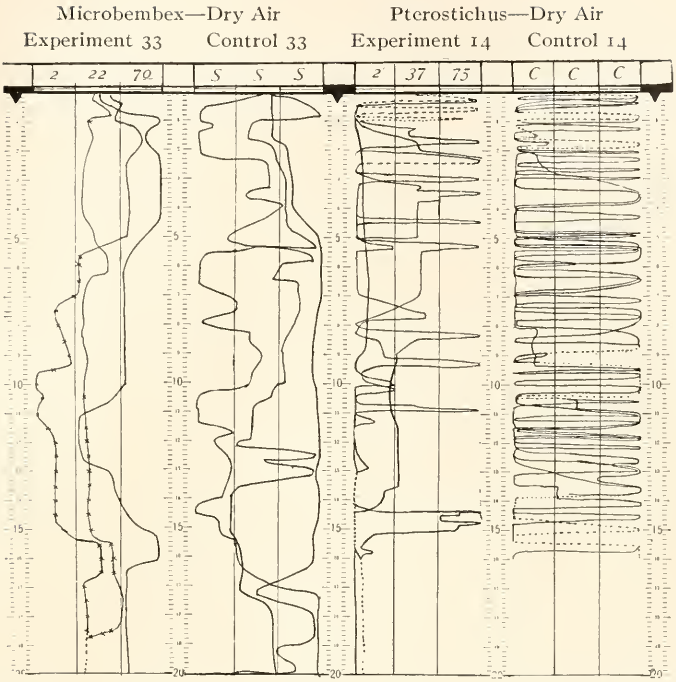

Chart V. Compares the reactions of a diurnal dune animal (*Microbembex*) and a nocturnal beech forest animal (*Pterostichus*). The two differ both in habitat preference and daily habits and the graphs are strikingly different. The crosses in tracings for *Microbembex* indicate that the animal was digging at the half minutes indicated opposite.

areas but they rested in one position, the depth of the cages not being sufficient to enable them to hop. The bronze tiger beetle (*Cicindela lecontei* Hald.) was tried and gave a negative reaction to air evaporating 1.2 c.c. in 20 minutes (3.6 c.c. per hour) and a positive reaction to air evaporating 0.52 c.c. per 20 minutes (1.56 c.c. per hour) produced by heat and current. They were so active that it was necessary to take readings of the number in each third every ten seconds.

2. *Rapidly Flow Air.*

The rate of evaporation is markedly influenced by rate of flow, and particularly there is a marked difference between a barely measurable movement and a very slight breeze such as .52 meter per second (1.1 miles per hour). Table III. brings out these relations in a rough way. More accurate results were not easily obtainable because of the difficulty of controlling humidity and temperature and accurately measuring the flows. It will be noted that a doubling of velocity was accompanied by a doubling of evaporation only in the cases of .052 and .104 meter per second. A breeze of .208 meter per second can be distinguished by the skin of the hand but usually lesser flows cannot.

TABLE III.

Showing the relation of evaporation to the rate of flow and to relative humidity under the experimental conditions, together with the relative rate of increase of evaporation and velocity. (*0.52* meter per sec. equals *1.1* miles per hour, *0.68* equals *1.5; 0.10* equals *0.2.*) The equipment is not accurate enough to make this more than a general guide. Pressure was not read.

Approximate Flow in Liters per Minute.	Approximate Velocity in Meters per Sec.	Approximate Evaporation in c.c. per Hour.	Temperature in Degrees C.	Relative Humidity in Per Cent. of Saturation.	Ratios.	
					Increase in Flow.	Increase in Evaporation.
1.9	.012	.25	22.4	50	1	1.0
3.9	.026	.40	22.2	53	2	1.6
7.8	.052	.75	22.2	53	4	3.0
15.6	.104	1.50	22.2	53	8	6.0
31.2	.208	2.00	22.2	54	16	8.0
62.4	.416	2.60	22.2	53	32	10.4
78.0	.520	2.90	18.8	45	40	11.6
15.6	.104	1.4	19.0	35	1	1.0
102.0	.680	3.7	19.0	35	6.5	2.5
15.6	.104	1.6	18.0	45	1	1
31.2	.208	2.3	18.0	45	2	1.4
Average:	.104	1.55	20.1	49	1	1.0
	.208	2.15	20.1	49	2	1.35

Compare with Schierbeck ('95), p. 221. Changes in rate of flow give greater difference in evaporation below .208 meter per sec. than above. Schierbeck's table gives accurate data from .88 m. per sec. to 4.23 m. per sec. for exposed water surfaces (see Livingston, G. T., '08, '09).

(*a*) *Physiological Effect and Reactions.*

The same species were studied and the same general physiological effects noted as where the difference in evaporation was due to dryness. Only slight evidence of mechanical stimulation occurred in the case of *Fontaria* in the gradient. The salamanders showed the same kind of activity and symptoms of drying in the rapid as in the dry air. The animals pushed

TABLE IV.

Showing the relation of animals to a gradient of evaporating power due to differences in rate of flow. Low evaporation was produced in two ways: (*a*) by allowing the moist air to drift across the cage at the rate of .026 to .052 meter per sec., marked "wet," and (*b*) by having no current at all, evaporation given as +.

No. of Experiment.	Species.	EXPERIMENT.												CONTROL.					
		Evaporation in c.c. During Experiment.			Relative Humidity in Per Cent. of Saturation.			Turned Back From		Per Cent. of Time in Thirds.			Temperature of Experiment.	Turned Back From		Per Cent. of Time in Thirds.			Temperature of Control.
		Low.	Medium.	High.	Low.	Medium.	High.	Low.	High.	Low.	Medium.	High.		Corresponding to Low.	Corresponding to High.	Corresponding to Low.	Corresponding to Medium.	Corresponding to High.	
50	*Plethodon cinereus*	+	0.15	0.45	47	47	47	0	1	87	5	8	21	1	0	31	31	38	21
71		wet	.60	0.97	98	56	56	4	8	72	27	1	24	0	1	0	32	68	23
Average:			.37	0.71		52	52	2	4.5	80	16	4	22.5	0.5	0.5	15	32	53	22
51	*P. glutinosus*	+	.22	0.45	66	66	66	0	2	79	10	11	20	4	2	33	18	49	19
52	*Rana*	+	.22	0.45	66	66	66	0	1	19	76	5	20	0	0	33	1	66	19
70		wet	.60	0.97	98	56	56	0	0	65	20	15	24	0	0	61	31	8	24
Average:			.41	0.71	—	61	61	0	.5	42	48	10	22	0	0	47	16	37	21
48	*Fontaria*	+	.37	1.05	47	47	47	0	1	37	27	36	22	0	0	32	30	38	22
48A								0	1	53	15	32	22	0	0	32	30	38	22
48B								0	0	40	18	42	22	0	0	32	30	38	22
Average Expt. 48:								0	1	43	21	36	22	0	0	32	30	38	22
69	*Fontaria*	wet	.60	0.95	98	56	56	0	3	46	26	28	24	1	1	44	14	42	23
Average:			.49	1.01		51	51	0	2	45	23	32	22	2	2	38	22	40	22.5
47	*Bufo*	+	.37	1.05	47	47	47	4	10	44	26	30	22	9	10	26	23	51	22
68		wet	.60	0.97	98	56	56	9	5	71	8	21	24	14	10	22	37	41	25
Average:			.42	1.01		51	51	6.5	7.5	57	17	26		11.5	10	24	30	46	24
49	*Geolycosa*	+	.37	1.05	47	47	47	9	9	30	25	45	22	8	8	28	30	42	22
67		wet	.37	1.05	98	47	47	14	9	31	23	46	22	0	3	36	40	24	23
Average:			.37	1.05		47	47	11.5	9	31	24	46	22	4	5.5	32	35	33	22

against the glass cover more often than in the dry air. This occurred in the cases of *Geolycosa* and the salamanders. Table IV. shows a greater degree of positiveness for moist than for still air (see Charts I. to IV., pp. 87, 90, 91, 95, central graphs).

3. *Warm Air.*

Table V. shows the effect of raising the temperature upon the humidity and evaporation. The difficulty in manipulating this sort of experiment lies in the fact that the atmometers and the water in the burettes *should be at the same temperature as the air used,* if results comparable to those at room temperature are to be obtained. This was accomplished approximately, for the purpose of obtaining the data presented in Table V., but the equipment was faulty and the table is probably only sufficiently accurate to give a general idea of the effect of raising the temperature. A rise of 15° to 17° C. is required to double the evaporation.

Table V.

Showing the effect of raising the temperature upon humidity and evaporation under the experimental conditions. Air pumped from a dry greenhouse. Flow *15.6* liters per min.; velocity over the evaporimeters about *.104* meter per sec. or 0.2 mile per hour.

	Unwarmed Air.			The Same Air Warmed.			
Date.	Temperature in Degrees Centigrade.	Humidity in Per Cent. of Saturation.	Evaporation in c.c. per Hour.	Temperature in Degrees Centigrade.	Temperature Increase.	Relative Humidity in Per Cent. of Saturation.	Evaporation in c.c. per Hour.
Dec. 20	19	37	1.4	30.0	11.0	17	2.0
Jan. 21	14.5	38	1.5	25.5	11.0	19	2.3
Jan. 24	22.6	37	1.6	27.5	5.0	25	2.3
Jan. 24	22.6	37	1.6	32.6	10.1	18	2.7
Jan. 24	22.0	32	1.6	38.0	16.0	14	3.1

(*a*) *Physiological Effect and Reactions.*

The effect of increased evaporation due to rise of temperature is more marked than when the evaporation is due to movement or to dryness. This is probably due to the fact that animals are quite sensitive to temperature alone although the temperature of either air or water has rarely been varied alone and a part of its supposed effect may be due to increased evaporation in air or to decrease in gases in solution in water.

TABLE VI.

Showing reaction to evaporation due to higher temperature. Flows approximately *15.6* liters per min. This type of experiment is more difficult than the others and the temperature, humidity and evaporation records are less accurate because the experiments were largely performed before the methods of control were perfected. Averages of controls are averages of all controls including those in the preceding tables.

No. of Experiment.	Species.	Experiment.											Control.								
		Evaporation.			Humidity.			Turnings From		Per Cent. of Time in			Temperature. Expt.		Turnings From			Per Cent. of Time in			
		Moist.	Medium.	Warm.	Moist.	Medium.	Warm.	Moist.	Warm.	Moist.	Medium.	Warm.	Warm.	Cool.	Control.	Corresponding to Moist.	Corresponding to Warm.	Corresponding to Moist.	Corresponding to Medium.	Corresponding to Warm.
54	*P. cinereus*...........	0.02	0.35	0.55	98	61	26	0	7	43	48	9	32	22	22	5	6	56	30	14
64		0.02	0.35	0.75	98	40	35	1	5	88	8	4	32	22	22	0	1	61	22	17
Average:		0.02	0.35	0.65	98	50	30	0.5	6	65	28	7	32	22	22	2.8	2.5	36	28	36
73	*Rana*.................	0.02	0.45	0.60	98	43	34	0	6	93	5	2	31	22	26	1	1	9	52	39
77		0.02	0.45	0.60	98	43	36	2	4	35	46	19	29	24	24	0	2	36	30	34
Average:		0.02	0.45	0.60	98	43	35	1	5	65	25	10	30	23	25	1	1	36	30	34
75	*Fontaria*..............	0.02	0.30	0.52	98	44	43	0	6	69	22	9	32	22	24	1	2	38	23	39
55		0.02	0.45	0.65	98	61	29	1	6	66	29	5	29	23	23	1	0	42	16	42
Average:		0.02	0.37	0.56	98	52	31	0.5	6	68	25	7	30	22	23	1.2	0.9	39	21	40
72	*Bufo*.................	0.02	0.45	0.60	98	43	36	0	3	87	1	12	29	23	22	13	9	21	45	34
74		0.02	0.45	0.60	98	43	36	9	13	36	31	33	29	23	24	8	15	25	23	52
76		0.02	0.45	0.70	98	43	34	24	28	51	22	27	29	24	24	13	13	21	32	47
Average:		0.02	0.45	0.60	98	43	36	16	14	58	18	24	29	23	23	8.0	8.1	28	34	38
65	*Geolycosa*.............	0.02	0.30	0.80	98	40	35	9	9	16	32	52	29	23	22	1	3	30	20	50
79		0.02	0.45	0.60	98	43	36	15	14	38	23	39	29	23	23	1	1	35	29	36
Average:		0.02	0.37	0.70	98	41	35	12	12	27	28	45	29	23	23	1.8	1.8	29	29	42

Plethodon cinereus is quickly affected and over-stimulation and coiling appear within one minute while in the dry and rapidly moving air from two to ten minutes are required to bring about the same result. All the activities and symptoms of loss of water are the same as in the dry air (p. 86). In the gradients (Table VI.) one of the graphs shows less clear avoidance of the hot air due to over-stimulation and some loss of correlation in movement. Otherwise the reactions were entirely similar. No temperature experiments were performed with *P. glutinosus* but the difference in behavior in hot and dry air in the tubes was comparable to that of *cinereus*.

Rana behaved exactly as in the dry air in the experiments, with only five degrees increase, but the graph of the gradient experiment with ten degrees difference is like that of *Plethodon* as a very clear orientation occurred.

Fontaria showed the greatest difference when compared with the dry air. The activity was much greater in the hot than in the dry or moving air. Movement in the tubes was increased six times with an increase of 8° C. In the gradient a distinct orientation occurred, the animal turned back upon encountering the hot air. This was a decided difference from the reaction to dry and moving air (compare graphs of Chart II., p. 91).

Some of the toads showed stimulation in the hot air. Activity was increased in the tubes and in the gradient experiments some of the individuals tended, to hop back and forth in the warm end (Chart II., Exp. 76) and only a weak negative reaction resulted. The detailed behavior of *Geolycosa* was not markedly affected by the difference in temperature and a slightly positive reaction was given. *Cicindela lecontei* was likewise positive in the gradient (see Table VII.) but in the tubes showed a greater tendency to "clean" the legs and antennæ while in the hot air.

4. *Death through Evaporation.*

All of the animals studied may be killed by loss of water. The results are given in Table VII. It will be noted that where records of size were preserved, the smaller animals died from loss of water much more quickly than the larger. This is perhaps due to the fact that the surface is greater in proportion to the

TABLE VII.

Showing the relative length of time and the relative amounts of evaporation required to kill the species used in the experiments.

Species.	No. of Experiment.	Method.	High Evaporation.			Medium Evaporation.			Low Evaporation.			Remarks.
			Died.			Exposed Died.*			Normal; After.			
			Evaporation in c.c.	Time in Minutes.	Length in cm.	Evaporation in c.c.	Time in Minutes.	Length in cm.	Evaporation in c.c.	Time in Minutes.	Length in cm.	
Plethodon cinereus ..	16	Dry.....	3.0	75	·	*1.4	160	·	0.7	160		
" " ..	16	"	2.3	65								
" " ..	82	"	5.5	65		1.0	60		1.5	60		
" " ..	97	"	1.7	40	4.2	*0.7	25	4.2	†0.2	25	4.0	† Experiment started at noon. Died 10 A.M. next day in the tube; no current.
" " ..	97	"	1.8	45	6.0	*1.2	45	6.0				
" " ..	97	"				*2.1	75	9.0				
	Aver	age	2.8	58		1.3	73					
P. cinereus........	97	Wind....	0.7	15		0.2	15		0.07	15		Medium did not die.
"	87	Hot (10°)										10 min. obs.; none killed.
"	88	"	0.5	23		0.1	20		0.0	20		
P. glutinosus.......	95	Dry.....	2.4	34	4 to 7							
"	98	"	5.4	160			*27					
	Aver	age	3.9	87								
"	93	Wind....	1.3	30								
"	89	Hot (8°)..	0.07	5								
Rana............	15	Dry.....	4.8	155	2	2.2	275	2	0.15	155	2	Lengths from nose to end of urostyle.
Bufo............	95A	"	5.4	160	5							
Pterostichus........	100	"	31.0	1,300						2,220		
Fontaria..........	100	"	38.0	1,800						2,220		
"	100	"	40.0	1,860						2,220		
	Aver	age *Font*·	39.0	1,830								
Geolycosa.........	100	Dry.....	42.0	2,220						2,220		

volume in the smaller animals. We note further that the animals die after a smaller amount of evaporation when the rate is slow than when it is more rapid. Since the loss in weight of the animals was not determined, it is impossible to state whether it is directly due to differences in the rate of evaporation from the animal and from the atmometer, or to concentration of the body fluids beyond a point compatible with life which resulted in death after a time no matter whether evaporation continued or not. It was noted that after the skin had become dry, the amphibians did not recover even when put into water. However the most remarkable fact brought out by the table is that the animals died more quickly from evaporation due to rapid movement of air than due to dryness. The same is true of evaporation due to higher temperature. *Bufo* survives longer than *Rana*. Of the arthropods *Pterostichus* (ground beetles), *Fontaria* and *Geolycosa* die in the order mentioned. The beetles died 22 hours after the beginning of the exposure.

V. General Discussion and Comparison.

The general problems involved in the results which have just been presented are among the more complex of physiology. The relations of the various species studied to their environments; the relation of kind of integument to survival time in dry air; and the question of reaction and irritability make necessary a rating of the different species and a discussion of the results of previous workers on the physiology of water starvation.

1. *Rating of the Species Studied.*

In order to make comparisons it is necessary to estimate the degree of avoidance of air of the high or low rate of evaporation. In the main there are two indications of reaction; (*a*) *time spent in the two kinds of air* (*halves of the cages*) and (*b*) *number of turnings back, upon encountering the avoided air*. When both are expressed in terms of per cent. of total and the two regarded as of equal value, ratings can be obtained as indicated in Table VIII. (Shelford and Allee, '13). Since the ratings are based upon a small number of experiments, they can be taken only as tentatively representing the relations of the animals to the rates of evaporation.

TABLE VIII.

Showing the rating of the different species studied when the turnings back from the modified air and percent of time in the two halves of the experimental cages are regarded as of equal value. The ratings are obtained from the percent of total turnings from the halves and the percent of time in the halves. The differences between the two percents in each case were added and divided by 2. When the greatest number of turnings is from the end in which least time was spent the turnings and time are of the same sign (+ or −).

Species.	Controls.		Experiments.						Average	
			Evaporation Produced by							
			Dryness.		Movement.		Temperature.			
	Number.	Rating.	No. Expts.	Rating.	No. Expts.	Rating.	No. Expts.	Rating.	No. Expts.	Rating.
Plethodon cinereus......	10	± 3.0	5	−71	2	−66	2	−82	9	−73
" *glutinosus*.....	2	± 7.0	1	−88	1	−82	—	—	2	−85
Pterostichus............	1	±11.0	1	−72					1	−72
Rana sylvatica..........	19	± 1.5	5	−68	2	−80	2	−69	9	−72
Fontaria corrugata.......	10	± 6.0	6	−43	4	−55	2	−83	12	−60
Bufo lentiginosa........	9	± 8.0	4	−46	2	−23	3	−27	9	−32
Microbembex...........	6	± 1.3	6	+ 6					6	+ 6
Geolycosa sp...........	7	±10.0	4	+18	2	+16	2	+12	8	+15

2. *Comparisons.*

(*a*) *Integument.*—An inspection of Table VII. shows that the animals killed by rapid evaporation fall into two distinct groups: (*a*) those dying with an evaporation varying from 0.07 to 5.40 c.c. after an exposure varying from five to one hundred and sixty-five minutes, and (*b*) those dying with an evaporation of 31.0 to 42.0 c.c. after an exposure of from 1,300 to 2,200 minutes. The first group is made up of soft-skinned amphibians, the second of chitin-covered arthropods. Even though the arthropods were much smaller (Hill, '06, p. 267) they lived from eight to four hundred and fifty times as long as the amphibians. The loss of water through a tracheal system should be at least as great as through lungs; the difference is probably primarily due to the character of the integument.

(*b*) *Reaction, Survival-Time, and Habitat Preference.*—A comparison of Tables VII. and VIII. shows that in general there is a rough relation between survival time and reaction among animals with *similar integuments.* Of the amphibians, the *Plethodons* died in dry air in 58 min. (*cinereus*) and 87 minutes (*glutinosus*) and are rated respectively at − 72 and − 85; *Bufo* died in 160 minutes and is rated at − 32 (compare charts I. and II.). Of the chitin-covered animals *Pterostichus* (Chart V.) is rated at − 72 (single experiment) and died in 1,300 minutes; *Fontaria* at − 60 died in 1,830 minutes; *Geolycosa* rated at + 15 died in 2,200 minutes (compare charts III. and IV.).

The ratings given in Table VIII. clearly fall into two groups which are habitat groups. The *Plethodons*, *Fontaria* and *Pterostichus* were taken from the surface of the ground under the leaves and in a primeval beech forest; *Geolycosa* and *Microbembex* (+ 6, see Chart V.) are regular residents of the driest open sand areas. The toad is an incidental resident of the sand area. A comparison of Tables VII. and VIII., shows that while a relation exists between habitat and survival time it is confined to animals with similar integuments. No such relation exists when one entire habitat is compared with the other habitat group. Omitting the toad, we find that the regular breeding residents of the two habitats (beech woods and open dunes) differ in sign and degree of reaction in a manner comparable with the difference in physical conditions of the habitats (Shelford, '12*b*). Distribution is then not a life and death matter for adults but a matter of behavior reaction (Shelford, '11, '12*a*, '12*b*; Shelford and Allee, '12*a*, '12*b*).

A further comparison of the different species given in the table shows important relations to vertical conditions of forest developmental stages (Yapp, '09; Sherff, '12; Shelford, '12*a*; Fuller, '12). The wood-frog spends much of its time during the day hopping about the forest floor. *P. cinereus* lives more of the time beneath the leaves and is clearly more sensitive to evaporation. *P. glutinosus* occurs in the beech woods proper in numbers only in moist seasons; ordinarily it is confined in ravines where Fuller ('12) found the average evaporation per day for the season to be 1.5 c.c. less than at the surface of the forest proper. Since *glutinosus* occurs in moister situations than does *cinereus*, the diference in the sensitiveness of the two species is related to habitat. The habits of *Pterostichus* are not well known; the species studied appear to be inhabitants of moist woods. *Fontaria* however while living under the leaves of the beech woods is most common in earlier forest stages (Shelford, '12) where the evaporation is 1.0 c.c. or more per day greater. It is clearly less sensitive to evaporation than *Pterostichus*. *Microbembex* and *Geolycosa* are confined to open sand situations.

3. *Physiology of Water Withdrawal and Water Starvation.*

Tiedeman ('36) described the symptoms of great thirst experienced by travelers in the desert. The first thirst is followed by dryness and smarting of the throat; next the respiratory action is increased and later long deep breaths alternate with hiccoughs; hoarseness occurs and is followed by loss of speech; the pulse is quickened; the skin becomes dry; the muscles become weak and a feeling of great fatigue ensues with staggering and labored movements. The thirst then becomes maddening and loss of consciousness usually follows. Hill ('06) states that with a loss of ten per cent. of his weight in water, a man usually dies. The study of the phenomena of water starvation dates from the beginning of modern experimental physiology. Some of the early experiments in physiology were water starvation experiments on birds and mammals. Schuchardt ('47, see Nothwang, '92*a*) found that pigeons fed on air-dry grain but deprived of water died in about eleven days. Scheffer ('52, see Nothwang, '92*a*, pp. 275–276) describes some of the symptoms of death by water starvation in pigeons. During the earlier part of the experiment there was great unrest and excitement accompanied by characteristic sounds. This gradually passed off and the animal became quiet and did not notice the surroundings or respond to stimuli. Northwang ('92) summarizes the earlier literature. He studied the dry weight of fat-free tissues of water-starved birds and found that it was increased. He came to the conclusion that death from want of water resulted from the accumulation of splitting products in the cells, due to the lack of sufficient fluid to remove them. The results according to this view should resemble fatigue, which may account for the fatigue symptoms which accompany water starvation. He states that fat animals resist lack of water better than those without fat because fat can be easily split to yield water. Pernice and Scagliosi ('95) worked upon the histology of fowls which had died of water starvation. They state that atrophy of the adipose tissue, muscles and abdominal organs occurs. There is also congestion and drying of the abdominal viscera. The authors found that the structural elements of the tissues, including the nervous system, had atrophied. They came to the conclusion that the possible water fluctuation of the animal tissues is very small and whenever a cell's water content passes a certain limit, death ensues.

All animals produce some water through the oxidation of the hydrogen in their food. According to Atwater (Hill, '06) man produces about one third to one fourth of the amount of water which he gives off through the skin and lungs. Mathews ('13) called attention to this fact in connection with the adaptation of reptiles to desert conditions. Burger ('07) studied the water relations of the meal worm (*Tenebrio molitor*) when kept in dry air and fed on bran which had been dried at 105° C. He considered that the animals were in essentially absolute dryness. Here they lived for weeks but lost weight. He found however that the per cent. of water in the animals remained practically the same until after death and came to the conclusion that the insect larvæ could not use their food to produce water and so the living substance itself was used. No doubt the food taken produced water but this was not sufficient in quantity. The most important fact brought out was that the per cent. of water remained about the same in spite of the extreme dryness and rapid loss of moisture.

No mechanism to prevent loss of water exists in the common frog; its water demand is supplied through the skin. Durig ('01) found that the common European frog died if the loss of water was rapid when 15 per cent. of the frog's weight was withdrawn. If the drying was slow the frogs could lose 30 to 39 per cent. of their weight in water without dying. When the weight was reduced to 61 per cent. the blood corpuscle count was increased to 2½ times the normal.

We note from the changes in activity due to withdrawal of water, inactivity brought out in the preceding pages, that there was usually increased activity (period of heightened sensibility) followed sometimes by erratic movements (period of over-stimulation), which was followed by depression, apparent fatigue (the depression period). The first and last periods were always evident. The second in some cases only.

(*a*) *Heightened Sensibility.*

The irritability of the animals is evidently increased by a small loss of water, as indicated by the periods of heightened sensibility and over-stimulation referred to on page 86. The same phenomenon is shown by the increasing avoidance of the air of high evaporating power after several entrances into this air. The increased sensibility is probably due to the concentration of the blood and tissue fluids. Dr. A. P. Mathews and Dr. Carlson have both informed me that when muscle preparations dry during the usual study, contractions and twitchings result. Clearly the drying of the surface of muscles may not only increase the concentration of the ions but may also interfere with neutrality. In the gradients and in the killing experiments the skin of the amphibians clearly dried slightly in the dry air. The same is probably true of the sense organs and tracheal surroundings of the arthropods. The CO_2 output may be interfered with by the drying (Krog, '03, '04; Winterstein, '12). An increased concentration of CO_2 may be a cause of the increased irritability, as Waller ('96) has found that a very small increase of this substance increases activity of nerves.

Carlson ('06) and Meek ('06) secured decreased vigor of contraction in heart and other muscle preparations by the withdrawal of water by means of sugar and glycerine solutions and increased it by diluting the isotonic solutions surrounding the preparations. The cause of the decreased irritability in the case of water withdrawal by hypertonic solutions, is not so apparent and an explanation is not so easily reached, especially when we consider the fact that *Paramœcium* gives the avoiding reaction when water is withdrawn by sugar solution (Jennings, '06). Still the water withdrawal may have been so rapid that an increase in irritability was overlooked because of its transitory character and only the depression period which follows noted (see below).

In the case of bark beetles Hennings ('07) found that dry air increased metabolism and some of their activities. This is probably true with reference to an optimum, as moist seasons usually favor insects. The problem is a complex one and much data must be accumulated before a solution can be reached. Headlee ('13) found that the rate of metabolism of bugs feeding on succulent plants was not increased or modified by variations in moisture.

(*b*) *Period of Over-stimulation.*

This probably results from the loss of skin action through drying, in the vertebrates. It took place in the amphibians when the skin became quite dry; it did not occur in the arthropods. Skin respiration is important in most of the amphibians, birds and mammals. Sheffer's pigeons passed through a period of unrest preceding inactivity.

(*c*) *Period of Depression.*

The period of depression came on gradually in the arthropods. In the case of *Plethodon glutinosus* when the experiments were continued for more than 20 minutes, the animals sometimes came to rest in an apparently fatigued state, in the medium or dry air and died in that position. Durig found that the irritability of the muscles ('01) was decreased (latent period increased) and that rate of conduction of nerves ('02) was decreased in the case of frogs that had lost from 8 to 30 per cent. of their weight in water.

4. *Importance of the Evaporation Rate.*

The work on the physiological effect of evaporation from the bodies of animals, has been confined chiefly to the warm-blooded domestic animals and man. The loss of water from the human body was early noticed by Hippocrates and by Galen. Chalmers (1776), Seguin and Lavoiser (1789–90), Abernathy (1793), and Sharling ('42) all appear to have noted water output from the body or lungs. Weyrick ('62) studied the loss of water from the body, Reinhard ('69) found that the water loss was dependent upon temperature, humidity, wind, velocity and pressure. These factors control evaporation (see also Falck, '72;

Erismann, '75). Rubner ('90*a*, '90*b*, '90*c*) found that the rate of evaporation was of much importance in connection with the factors pointed out by Reinhard, in determining the metabolism, and general heat regulation economy in men and dogs, and with Cramer ('94) the effect of hair covering and of sunlight upon water loss and heat regulation. Schierbeck ('95) discussed methods of measuring the effect of atmosphere upon organisms. He found that the evaporation varies as the fourth root of the wind velocity. His conclusions regarding the measure of climate have been borne out by later workers: "Bei der Beurtheilung des Einflusses eines Klimas auf die Wärmeregulirung des Organismus und bei der Beurtheilung der austrocknenden Wirkung desselben sowohl auf den Organismus als auf leblose Gegenstände ist das Hauptgewicht auf die Geschwindigkeit der Verdampfung zu legen." Wolpert ('98, '99, '02*a*, and '02*b*) studied the effect of moisture on laborers, the effect of oiling the skin on water loss, the influences of evaporation upon the skin, and the influence of air movement upon water loss and carbon dioxide production. Up to 25° C. the latter was increased; at higher temperatures decreased. Haldane ('05) worked upon the effect of high temperatures on man and found that the discomfort was due to a rise in the body temperature. The ill effects were partially prevented if the air was kept moving thus increasing the evaporation. Hill ('06) summarizes the important work on the subject of water relations and heat regulation. The heat regulating power of a mouse fails at 24–25° C. (p. 269) in a saturated atmosphere, due to rapid loss of heat, and the animals die from cooling. In man it fails at 29° C. in a saturated atmosphere and if he is active and clothed, he suffers from overheating; at 37° and in the absence of clothing any exertion is practically impossible. In a dry air a man may sit for a time at 100° C. Sutton ('08) states that heat stroke occurs only in a very moist atmosphere (see also Osborne, '10). Aron ('11) working on men and monkeys, found that death from exposure to the tropical sun in the Philippines was not due to any effect of the tropical light (Angstrom, '99; Woodruff, '05; Caskellani and Chalmers, '10), as had commonly been supposed but to an overheating of the body. This could be prevented by shade or by air currents which raised the evaporation. In conclusion he states: "My experiments demonstrate the enormous physiological and hygienic importance of ample water evaporation in the tropics."

Hill states that the increased blood count in mammals in high altitudes and balloon ascensions is due to the transudation of the lymph out of the peripheral vessels from which the sample is drawn. Cronheim ('12) however insists that loss of water through the lungs and through evaporation is the factor; no doubt both are correct in a measure. Reduction of pressure increases evaporation (Nothwang, '92).

While from the standpoint of irritability little has been done there is an excellent experimental basis for a statement of the factors controlling the distribution of warm-blooded animals. The importance of any factor on the distribution of animals is its importance in the life of the animals. From the literature cited and from other literature included in the bibliography it is evident that in the case of mammals temperature data have little significance unless the humidity is known. Neither of these can be interpreted without a knowledge of the pressure, isolation, and wind movement. The experimental foundation for the consideration of all these factors was clearly laid down by Reinhard ('69) and Rubner ('90). The best method of expressing them climatologically was stated by Shierbeck ('95) as the amount of water evaporated. This does not mean that records of the separate factors involved, namely, temperature, pressure humidity, isolation, wind movement, etc., should not be made but rather that the best expression of their combined action is the rate of evaporation.

The striking similarity of reaction and survival time to similar rates of evaporation on the part of the animals regardless of whether due to dryness, heat, or velocity speaks very strongly for the measure of evaporation in connection with cold-blooded animals. It is a noteworthy fact that the relation of warm-blooded animals to climatic factors had been observed (Livingstone,' 58) and experimentally studied (Reinhard, '69; Rubner, '90) before Merriam ('90, '94, '98) published his theory of temperature control (see Swain, '05; Craig, '08; Roosevelt, '10; Mathews, '13). He made a most important contribution in his emphasis of the breeding period. However there is no good evidence that total temperature above an arbitrary minimum is more significant than is total pressure, total sunshine, total wind movement, or (Walker, '03) total humidity. All must be considered together. Temperature control has "worked" in the mapping of distribution just as any theory whatsoever will work for some species be it concerned with a wandering pole or an Atlantis. The facts and causes of distribution are much more complex than the temperature control assumes. While all facts of distribution are worthy of explanation, the biological processes concerned are of vast importance for they include the most complex problems of biochemistry and life phenomena. The increase in irritability shown by the animals studied, the remarkable water-regulating power of the meal worms, brought out by Berger ('07), the quick regulatory changes of the mammals, and other responses to the physical environment (including the surrounding medium) open many new problems to the biochemist. Our explanation of the phenomena concerned must accord with the facts of relations in nature as well as with the results of the laboratory experiments. Experimental ecological investigations give promise of contributing as much toward the solution of some of the broader biological problems as will the investigation of subjects in fields where speculation has added interest and concentrated attention in years past, and, so far, given us a total progress of questionable significance.

Summary.

1. The animals studied react to evaporation whether it is produced by movement, dryness, or heat (p. 105).

2. The sign and degree of reaction are in accord with the comparative rates of evaporation in the experiments and in the habitats from which the animals were collected (p. 105).

3. The animals of a habitat are in general agreement in the matter of sign and degree of reaction; the minor differences which occur are related to vertical conditions, position of maximum abundance in succession, and kind of integument (pp. 97 and 106).

4. Short exposure to high evaporation increases sensibility to evaporation (pp. 87, 90, 91, 95, and 97 (charts)).

5. In the survival time experiments, heightened sensibility was sometimes followed by over-stimulation and always by depression and apparent fatigue (pp. 109–10).

6. There is a rough agreement between survival time and kind of integument but no agreement between survival time and habitat when a number of different members of a community are taken together (p. 106).

7. The rate of evaporation is the best index of the combined action of wind, temperature, isolation, and dryness of air.

8. Temperature is probably no more significant than moisture, isolation, or wind (p. 112).

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University of Chicago,
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SOME EFFECTS ON FUNDULUS OF CHANGES IN THE DENSITY OF THE SURROUNDING MEDIUM.

GEORGE G. SCOTT.

Bert, '71, found that certain teleosts changed in weight on being immersed in water having a different density from that of the normal medium to which they were accustomed, though in some cases death occurred before any considerable change in weight had taken place. Garrey, '05, found that 80 per cent. of *Fundulus heteroclitus* taken from sea water lived in fresh water for six weeks. Loeb, '00, said, "*Fundulus* can be thrown from sea water into distilled water without any considerable swelling or without any visible injurious effects." Sumner, '06, reported a series of experiments from which he concluded that *Fundulus* did not survive transference from sea water to fresh water. He also concluded that diluted sea water containing only 2 per cent. to 4 per cent. of the salinity of pure sea water had a salutary influence on the preservation of life. Since the osmotic pressure of fresh water is but little less than this diluted sea water and since *Fundulus* survived in this solution but not in the other, Sumner concluded that the question of survival or death is not a question of difference in osmotic pressures. Sumner also investigated the changes in weight undergone by *Fundulus* in different dilutions of sea water. He found that in fresh water there was a slight gain which was followed by a loss until near the normal weight. In general transference to a hypertonic solution resulted in loss of weight, while in hypotonic solutions there was noted a gain in weight. And yet the change in weight bore no constant ratio to the changes in the osmotic pressure of sea water. Sumner found indications of a smaller gain in *Fundulus* from Woods Hole than in those from the New York Aquarium. He accounts for this as being due to differences in the physiological state of the organism at the higher temperature of the summer months. Their more active metabolism at this period might account for the greater permeability. On account of the diverse position taken by Garrey and Loeb on the one hand and Sumner on the other the present author desires to publish observations on the changes in weight in *Fundulus heteroclitus* resulting from immersions in solutions differing in density from that of sea water.

Fundulus heteroclitus, although found in sea water and brackish waters, is known at times to pass into fresh waters. Quoting from Sumner, p. 56, we find, "Bean, '03, says of *heteroclitus* that it sometimes ascends streams beyond tide water"—"Smith, '97, states that it is often found landlocked in ice or quarry ponds." "Dr. H. M. Smith informs me that it is found permanently in the vicinity of Washington, in the Potomac and its tributaries and also in ponds."

It is thus abundantly established that *heteroclitus* is found in fresh waters as well as brackish waters and even sea water. This does not mean however that they will readily survive sudden changes from salt to fresh water or *vice versa*. Sumner tried the effect of acclimatization by transferring 25 *F. heteroclitus* from the sea water (sp. gr. 1.025) to fresh water reducing the salinity by .001ths, hourly. At the end of thirty days but eleven were alive. From this Sumner concluded that complete acclimatization failed. Of course this is true since fourteen were dead. But what of the survivors? Why was not complete individual acclimatization exhibited in the cases of these? With regard to acclimatization Eugene Smith, '12, says that *Fundulus heteroclitus* may be transferred from salt and brackish to fresh water. They may be transferred more safely, the less degree of salinity there is in the water from which they come. Furthermore, while very few of those transferred from salt water directly to fresh survived the sudden change, an increasing number survived of those gradually transferred in the course of a week or two through a number of changes of water. My records show that such fishes lived from four to six months up to two years—one lived over three years." The above probably represents the truth of the matter. It will be observed here that the above writer emphasizes the suddenness of the transfer. The effect of a stimulus is related to the suddenness of its onset. In fact the living mechanism may be injured under too sudden as well as strong stimuli.

On July 10, 1908, I transferred ten *Fundulus heteroclitus* through a series of changes from sea water to a large rectangular dish containing about 20 liters of fresh tap water. In the bottom of the dish was placed some gravel from the shore near by—the gravel having been thoroughly washed in tap water. The water in the tank was completely changed every day or so—and once a week the tank was thoroughly cleaned. Food was placed in the tank daily, but all uneaten portions were removed after a few hours. On the whole the fishes did not seem eager for the food. Most of the fishes died during the next three or four weeks but one fish was alive and apparently in good condition on September 8, 1908, sixty days after the beginning of the experiment. A rough test of the water toward the end of the period showed a slightly greater amount of chlorine than was present in fresh water. And yet the hydrometer recorded a specific gravity of about 1.000. At first we might think that Sumner's contention as to the life-saving action of a small amount of salts was borne out here. But the amount of salts in which this specimen survived was less than that claimed by Sumner as necessary to exert a life-saving action. At the same time I do not wish to disclaim Sumner's contention. I only wish to point out a case where this conclusion does not follow.

Not only will *Fundulus* survive transfer to fresh water, but regeneration of removed tissues takes place under these conditions. This is shown by the results of the following experiments carried out with *Fundulus heteroclitus* taken from the New York Aquarium from the diluted harbor water which had a specific gravity of about 1.012 (sea water having a sp. gr. of 1.025). The caudal fin was removed in the same manner as described in a former paper by the present author (Scott). After the removal of the fin the fishes were transferred gradually to fresh water in a large rectangular jar which was constantly aerated from the compressed air supply in the laboratory at the College of the City of New York. The water was siphoned off nearly every day and replaced by a fresh supply from the tap. Fresh-water plants were kept in the experimental aquarium during the latter part of the period. The specimens were fed with fish food which they soon learned to take. I was not concerned at the time with the question as to the percentage of *Fundulus* that survive transfer to fresh water. The experiment was begun on March 13, 1909. On April 2, twenty days after, ten survivors were removed. The total body length was taken together with the length of the regenerated tissue of the caudal fin. The average actual length of the regenerated tissue of the caudal fin was 0.238 cm. The remaining ten survivors were removed on April 16, thirty-four days after the experiment was begun. The average actual length of regenerated tissue was .495 cm. The temperature of the water was about 20° C. A second experiment was begun on November 18, 1910. Out of thirty fishes operated on and placed in fresh water on November 18, but seven survived. In forty-two days during which the fish lived in fresh water the length of regenerated tissue was .41 cm. Out of a lot of thirty other specimens operated on two weeks later six survivors on December 30, 1910, about twenty-eight days after the experiment was begun, showed a regeneration of .23 cm. During the course of the experiment the fishes were transferred every few days to a smaller glass jar and the aquarium thoroughly cleaned and replenished with fresh water from the tap. In 1908 I found that the average length of regenerated caudal fin tissue of 108 specimens of *Fundulus heteroclitus* in sea water at Woods Hole for a month was about 0.6 cm. It will be seen that in fresh water the regeneration of the first of the above lots was 0.495 cm. in 34 days, that of the second lot, 0.41 cm. in 42 days. It appears probable that in fresh water the amount of regenerated tissue is decreased but because the animal as a whole is affected by fresh water. Further discussion is not proper on account of the meagerness of the data. Results show that not only does *Fundulus* survive transfer to fresh water but also that under these conditions, physiological processes are sufficiently normal for the regeneration of removed tissue.

Sumner in his practice in using numbers for fishes, found that frequently individuals died and so the carrying on of the experiment was interfered with. I determined therefore to study the changes in weight in *Fundulus* in solutions differing in density from that of sea water, by keeping a record of the individual changes in weight. Now since some specimens of *Fundulus* die sooner than others when transferred from sea water to fresh water, and since an increase in weight occurs when they are thus treated, the idea occurred to me as to whether the change in weight is less for the surviving individuals than in the case of those dying. Accordingly a number of experiments were tried with individuals transferred to separate dishes each containing a liter of fresh water. Each specimen was rinsed in fresh water, the free water absorbed on a soft clean towel, the specimen weighed and then placed in the dish indicated above. After a certain period the specimen was again similarly weighed and replaced in the dish with fresh water again. As a check a number of specimens were reweighed immediately. The dish in which they were weighed was also weighed after the fish was removed and compared with its former weight. No fish was handled more than was absolutely necessary. In fact handling was limited to picking the fish from the towel, placing it in the experimental dish.

Difference of opinion exists as to the effect of removing the scales or injuring the skin. For example, Bert found that the removal of mucus from the skin of the eel caused its death in sea water—where it would otherwise survive this transfer. He found that the eel survived transfer from fresh to salt water and was surprised when in a similar experiment carried out by his assistant, the eel died. He learned that the assistant had unconsciously removed mucus from the skin of the eel in the struggles involved in making the transfer. The experiments of Garrey corroborated Bert. Removing the scales or skin from portions of the surface of the body resulted in the rapid death of *Fundulus heteroclitus* on being transferred to fresh water, or to sea water, but of those kept in diluted sea water, approximately isotonic with the blood, none had died at the time the others were dead. Sumner obtained opposite results for in an experiment which he carried out at Woods Hole; he transferred *Fundulus heteroclitus* to sea water full strength or to diluted sea water having a sp. gr. of 1.001. He found that most lived although the skin had been removed from one entire side of the fish. In fresh water all these fish were dead in a few days. Sumner called attention to the well-known fact that hardy species survive mutilations of the body surface. In fact in experiments which he carried on at the New York Aquarium he noted the beginning of regeneration of scales six days after they had been removed, and during which they had been kept in sea water with a specific gravity of 1.025 having been transferred to this from the dilute harbor water with a density of 1.007. Mr. Denyse, of the New York Aquarium, informs me that it has been his experience that death usually accompanies serious injury to skin of fishes. Notwithstanding the difference of opinion cited above it is true nevertheless that in none of the experiments about to be described were scales removed. In fact, there was no visible evidence of the coat over the scales being injured. Attention is here called to a further consideration before the experiments are described.

The specimens used in the following experiments bore no surface abrasions and to the eye appeared normal. They were taken as needed from a large storage tank supplied with running sea water to which tank new specimens were added by the collector from time to time. Not only was a rough selection employed here but a further examination was made before they were used in the experiment. 153 specimens were used in 21 experiments and 988 weight determinations were made.

Control experiments with *Fundulus* in sea water showed on the whole a slight loss in weight as time went on. The results were similar to those found by Sumner. Certain individuals died in this control experiment showing that death in the following cases is not always due to the experimental solutions. In an experiment with six fishes in which an individual record of each was kept the entire lot was dead about eighteen hours after the experiment began. Variations in the individual weights were evident. But since they were all dead at the end of the period in question the average results will alone be given. Thus the average gain in weight at the end of two hours was 5.2 per cent.; at the end of five hours, 8.7 per cent.; at the end of eighteen hours, 15.7 per cent. In a second lot, the results were similar, five of a lot of six specimens were dead at the end of twenty hours. The change of weight was as follows, at the end of three hours 4.4 per cent.; at the end of five hours, 7.3 per cent.; at the end of seven hours, 9.6 per cent. This is the average of five specimens. The sixth was dead and showed a gain of 12.4 per cent. The average of the other five, all dead at the end of twenty hours, was an increase in weight of 19.0 per cent. In a third experiment it was clear that an increase in weight was greater in some specimens than in others. While all were dead at the end of twenty-three hours, yet three of the nine experimented with were dead at the end of nine hours. Three others appeared "sick"; they would swim on the side, turn over, follow the bottom, then start to swim again. The gain in weight in these was above that of the others still alive. In other words the results of these three experiments indicated that where there was an excessive gain in weight this was followed by early death. A comparison with these results with those of a fourth experiment about to be described in detail demonstrates the difference in different lots of fishes which in external appearance seemed normal. In this fourth experiment lasting over two days during which eleven weight determinations were made of nearly every specimen this differentiation was all the more striking and because of this, the experiment will be described in more detail.

The actual weights are not given. The table gives the percentage change in weight at each period which is found by comparing the weight of each specimen at the end of the period in question with the original loss in weight. The sign "+" means a gain in weight, while the sign "−" means a loss in weight. The results of this experiment are shown in Table I. The first noticeable feature in this record is the individual variation in the change in weight in all of the specimens. At the end of the observational period seven are dead and eleven are alive; while one is recorded as being lost. All of those alive at the end of the experiment weigh less than they did at some period after the beginning of the sojourn in fresh water. Indeed five weigh less than they did at the beginning of the experiment. If the changes in individual weight be followed, it will be seen to be a case of ups and downs in weight increases. All the specimens show a gain in weight during the first period. In some the gains are greater than in others. Specimen 5 with an initial gain of 8.0 per cent. recovers from this and later loses in weight and is alive at the end of the period. Specimen 18, on the other hand, shows a gain of 2.1 per cent. at first but this increases steadily

Table I.

Showing the Percentage Change in Weight in Individual *Fundulus heteroclitus* at Different Times During Immersion in Fresh Water.

Periods.	1	2	3	4	5	6	7	8	9	10	11	
Hours.	1+	3	4	18+	20+	22+	24	40	46	47	54+	
1 =	+3.1	+5.2	+5.0	+2.7	+5.6	+3.1	+3.8	+0.4	+1.4	+2.7	−2.9	(alive)
2 =	+2.2	+3.5	+0.4	+0.9	+0.3	+1.6	+1.8	+0.5	+0.9	+3.5	+0.3	"
3 =	+1.5	+1.9	+2.3	+1.9	+2.6	+2.4	+2.0	+0.9			+1.5	"
4 =	+2.9	+4.8	+4.5	+4.1	+3.7	+4.8	+4.4	+1.7	+5.7	(lost)		(lost)
5 =	+8.0	+9.5	+6.6	+7.8	+6.3	+8.1	+8.3	+3.0	+2.7	+1.4	−1.4	(alive)
6 =	+2.7	+4.3	+5.4	+7.1	+5.6	+6.4	+5.6	+3.1	+5.4	+5.6	+12.1	(dead)
7 =	+3.3	+4.0	+0.9	+4.3	+2.3	+1.5	+0.8	+1.0	+2.9	+3.8	−5.8	(alive)
8 =	+2.3	+3.6	+2.2	+1.2	+3.9	+1.0	+2.2	+1.2	+1.7	+3.7	+2.7	(alive)
9 =	+1.7	+2.7	+1.1	+1.2	+2.2	+1.9	+2.1	+1.2	+0.3	+3.9	+3.7	(alive)
10 =	+1.0	+3.4	+9.3	+14.1	+17.0	+17.8	+20.9	+28.0				(dead)
11 =	+2.3	+3.3	+8.6	+11.5	+15.2	+17.5						(dead)
12 =	+3.7	+4.3	+1.1	+9.2	+10.3	+12.0	+18.7					(dead)
13 =	+1.8	+2.7	+2.8	+2.1	+4.3	+1.4	+2.7	+2.3	+1.4	0	+0.5	(alive)
14 =	+1.9	+3.4	+4.9	+4.0	+5.7	+4.5	+4.6	+3.4	+2.7	+1.5	+0.2	(alive)
15 =	+1.4	+3.3	+2.0	+2.7	+2.9	+3.1	+2.0	+1.1	+2.0	+1.4	−9.6	(alive)
16 =	+4.4	+3.4	+6.1	+7.6	+8.0	+9.9	+9.2	+20.2				(dead)
17 =	+2.0	+3.4	+2.9	+2.7	+3.0	+3.4	+2.9	+3.3	+4.2	—	+16.4	(dead)
18 =	+2.1	+4.0	+8.0	+8.7	0	+10.3	+10.6	+16.7				(dead)
19 =	+5.1	+2.8	+1.2	+0.3	+0.7	+0.4	+0.2	+1.6	+2.7	+3.5	−4.9	(alive)

until it is dead at the end of the forty-hour period having shown a gain of 16 per cent. It is well known that fishes absorb water and increase in weight after death. Some of the final weight increases in the dead specimens may have been due to post-mortem processes. But there is ample evidence that the increase in weight began before death. This is shown by specimens 10, 11, 12 and 18. On the whole, selection takes place when *Fundulus* is placed in fresh water. This experiment shows at least one phenomenon accompanying the acclimatization of *Fundulus* to fresh water. As was said above, it is now well known that when *Fundulus* is put in fresh water from sea water, that some die and some survive. The present experiments show that the survivors after the first gain weight incidental to being placed in fresh water, recover from this gain, lose weight and survive. Others either at the beginning or later begin to steadily increase in weight. This may be sudden and at any time during the course of the experiment. In another experiment, where *Fundulus* was placed in a solution of one half sea water plus one half fresh water, some specimens gained somewhat as the survivors in the present experiments. They later lost. Others showed a slight loss from the beginning. Those that died resembled those that died in sea water, that is, the changes in weight gave no clue to the cause of death. The effect of this diluted sea water is not one half the effect of the sea water. The osmotic pressure of the diluted sea water is about half that of full strength sea water. In other words the effect of the two solutions is not proportional to the differences in their osmotic pressures. The result is similar to that found by Sumner, '05. Some of the specimens in the diluted sea water died but showed no peculiar weight differences.

Finally individual records were kept of changes in weight when fishes of this same species were placed in sea water to which known quantities of sea salt had been added. These experiments also revealed great variations in weight of one individual as compared with another. As would be expected the survival time decreased as the strength of the solution increased but not in proportion to the increase in the density of the surrounding medium. The results of some of these experiments are shown in Table II. The specimens lost in weight in these hypertonic solutions. The fishes in solution "D" are dead in four hours and lost but 9.5 per cent. of their weight while the fishes in the less dilute solutions "A," "B," "C" show a much greater loss

Table II.

A = Showing the Changes in Weight in *Fundulus* in a Solution of Seawater to Each Liter of which was added 16 Grms. of Sea Salt.

Periods.	1	2	3	4	5	6	7	8	9
Hours.	3+	7+	22+	27+	43+	54+	67	91	118+
1 =	−6.3%	− 7.6	−15.4	−14.8	− 9.7	−13.7	(dead)		
2 =	−9.6	−16.1	−14.4	(dead)					
3 =	−2.7	− 6.2	− 3.4	− 3.4	− 1.6	− 1.8	−1.3	−2.0	−0.9
4 =	−3.3	− 5.3	− 3.7	− 4.0	− 8.6	− 8.6	(dead)		
5 =	−4.2	− 4.9	− 9.7	− 8.7	−16.5	(dead)			

B = Changes in Weight in Solution of Seawater to Each Liter of which 22 Grms. of Sea Salt was added.

Periods.	1	2	3	4	5
Hours.	3+	6+	8+	22+	
1 =	− 9.4%	−12.6	−13.2	−17.9	(dead)
2 =	− 9.6	−13.7	−14.9	−19.3	"
3 =	− 6.0	−17.7			"
4 =	− 9.9	−14.7	−16.1	−17.0	"
5 =	−10.0	−13.6	−13.9		"

C = Changes in Weight in Solution of Seawater to Each Liter of which 42 Grms. of Sea Salt is added.

Periods.	1	2	
Hours.	3+	6	
1 =	−12.4%	−15.5	(dead)
2 =	−12.5	−15.9	"
3 =	− 9.7	−13.4	"
4 =	− 8.4	−11.0	"
5 =	−11.8	−17.6	"

D = Changes in Weight in Solution of Seawater to Each Liter of which 62 Grms. of Sea Salt was added.

Periods.	1
Hours.	4
1 =	− 9.6% (dead)
2 =	− 8.1 "
3 =	− 7.9 "
4 =	− 8.7 "
5 =	−12.5 "

in weight and after a longer time. This rather sudden death of these in solution "D" accompanied by a smaller loss in weight as compared with the others suggests that in these hypertonic experiments death may be due to the direct action of the excessive amount of sodium chloride present in solution. Still there are evidences of the ability of the organism to react against even this condition for in one experiment in which the fishes were kept in a solution of sea water to which 16 gms. of sea salt were added per liter, most of the specimens were dead after two days showing a loss in weight. One (no. 3) lived for nearly five days and showed in its weight determinations fluctuations similar to the survivors in fresh water although of course in this case they were of an opposite nature due to the hypertonic solution. Mather, '81, suggested that the cause of death of salt-water fishes in fresh water was not due so much to chemical differences as to differences in osmotic pressures. Sumner takes the opposite view. But both may play a part in producing the results. It is possible that *Fundulus* resembles the eel, *Anguilla*, and undergoes a reduction of the osmotic pressure of its blood after sojourn in fresh water. Dakin, '08, found that in sea water the blood of *Anguilla* resembled that of marine teleosts, though not quite as great. In fresh water, the osmotic pressure of its blood was similar, though not quite as low as that of the blood of fresh-water teleosts.

Sumner found that a loss of salts took place when *Fundulus* is placed in fresh water and that the amount lost decreased as the time of sojourn in fresh water increased. This result also indicates that a decrease in the permeability of the limiting membranes of the body takes place due to the change in the environment.

At least two positions can be taken with regard to this matter. In the first place however the question arises as to the part of the body concerned in the effects noted. Sumner gives experimental evidence based on studies with teleosts to the effect that the gill membranes are the structures concerned. Experiments of my own with elasmobranchs show the same results. With regard to the two possible views to take, one is, that the kidneys regulate the osmotic pressure of the blood when the organism is immersed in fresh water, eliminating the excess of water taken in through the gill membranes. But what of the constant effect of this excessive work on the kidneys in the case of specimens successfully transferred from sea water to fresh and living for a number of years in fresh water, as Smith has described. Such a view is manifestly weak. There is but little doubt that the changes brought about in the organism due to immersion in fresh water are due to diffusion of water through the gill membranes, and that the permeability of the gill membranes is changed. A second view as to what subsequently happens is that the gill membranes after this attack are modified. And in such a process must not other parts of the organism be concerned? The gill membranes cannot be changed of themselves. They are under the influence of the nervous system. Blood carries materials to the membranes. The experiments show analogies to the response of animals to the inoculation of bacteria, a period of ups and downs, recovery in the case of some, death in others. It seems to me that views which place the emphasis upon the influence of the external agents on the membranes and explain acquired impermeability as being due to some sort of tanning action, are incomplete. What kind of respiration could take place through tanned membranes? Evidence has been obtained from the plant world to the effect that plant cells can modify their permeability. It is not necessary, however, to agree with Philip, '10, when he says, "This fact and others which have just been quoted, will serve to show that a purely physical theory of the exchanges which take place across a living membrane is inadequate; there is a physiological permeability as well as a physical permeability." This is but begging the question.

LITERATURE LIST.

Bert, P.

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THE SPERMATOGENESIS OF A DAPHNID, SIMOCEPHALUS VETULUS.

A Preliminary Paper.

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In Daphnids several generations of parthenogenetic females are followed by a sexual generation in which males are produced together with eggs requiring fertilization in order to develop. These eggs, when fertilized, hatch invariably into females which serve as stem mothers for a fresh series of parthenogenetic generations.

A study of the spermatogenesis of a Daphnid would therefore be of special interest in deciding the question regarding the specificity of spermatozoa in the determination of sex.

During the summer of 1912 males of *Simocephalus vetulus* were obtained in abundance from the fresh-water pools in the vicinity of the Marine Biological Laboratory at Woods Hole, Mass.

The liquid of Petrunkewitsch and Flemming's fluid (strong) proved to be the best fixing agents for the purpose.

The sections were stained with Heidenhain's hæmatoxylin and also with safranin and light green.

The immature testis is an elongate body consisting of a solid mass of spermatogonial cells with ill-defined boundaries and enclosed in a thin membranous wall which is carried out posteriorly as the vas deferens.

The spermatogonial cells are fairly uniform in size. Their nuclei are large and contain one or two nucleoli (Fig. 3, *a*).

A constant feature is the presence, here and there throughout the testis, of cells distinguished by their disproportionately large nuclei and nucleoli. They occur in all sizes varying from those of ordinary spermatogonia to giant forms shown in Fig. 1.

In more mature testes numerous regions or islands are discernible especially in the center in which cells may be found in various stages of maturation, the cells of an island being in about the same stage.

As the cells in these islands advance in development a ring of undifferentiated cytoplasm remains about each island. This cytoplasm is continuous with that surrounding other islands and also with the cytoplasm of the giant cells (Fig. 2), which lie between the islands.

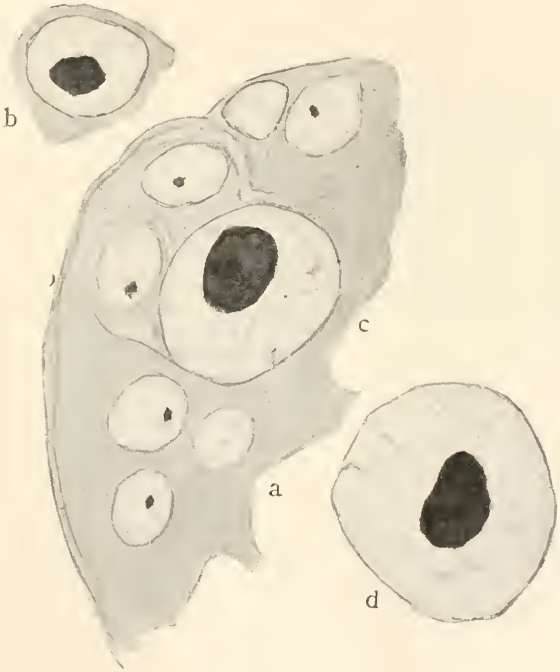

Fig. 1.

The islands remain distinct until their contents are transformed into free spermatids. The cytoplasm between the islands then disintegrates, producing large spaces in which lie the free spermatids. The testis thus acquires a lumen which ever increases in size until the testis becomes a hollow sac full of ripe sperm.

In the last stages, when the testis is a hollow sac whose walls consist merely of an epithelial coat, the giant nuclei are not to be found. However, there may be seen, scattered among the free sperm, irregular particles of disintegrating cytoplasm, possibly the remains of the rings of cytoplasm which formerly enclosed the islands.

The giant nuclei attain their greatest size in testes which are composed almost entirely of islands of spermatocytes in the various stages of maturation but in which no lumen yet exists.

Simultaneously with the growth of the giant cell the nucleus and noticeably the nucleolus increase in size. A change also takes place in the staining reaction of the nucleus. The chromatin network, which hitherto together with the nucleolus stained red with safranin, loses that capacity and takes up light green, an acid stain.

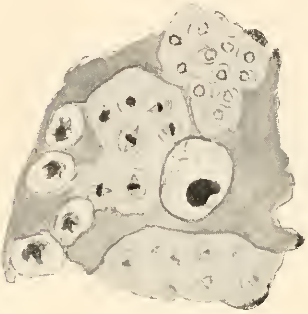

FIG. 2.

A well-grown giant cell thus possesses a large nucleus with an enormous basic staining nucleolus and an acid staining nuclear network, the granules in the surrounding cytoplasm staining red with safranin.

Similar cells have been described, in literature on spermatogenesis, as rudimentary ova. A significant fact, however, which militates against such an interpretation, at any rate for the giant cells in the *Simocephalus* testis, is that they grow directly from spermatogonia and do not pass through the synapsis stage.

The striking but superficial resemblance between these giant cells and growing oöcytes is evidently due to the one function common to both, viz., that of an enormous growth in size.

The ever-increasing size of the nucleolus during growth and its final dissolution in both types of cells favors the assumption that the nucleolus is intimately connected with cell growth.

In the spermatocytes in *Simocephalus* where no growth occurs the spermatogonial nucleolus remains small during synapsis and early disappears. The same is true for *Pandarus*[1] and for *Cyclops*.[2]

On the other hand, in spermatocytes where growth does occur, a growing nucleolus is described by Schmalz[3] in an Ostracod. In this form the nucleolus grows during synapsis and during the subsequent growth period to disappear on the formation of the spindle for the first maturation division.

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[1] J. F. McClendon, *Arch. f. Zellforsch.*, V., 1909.

[2] R. Chambers, Jr., Univ. of Toronto Studies, Biol. Ser., No. 14, 1912.

[3] J. Schmalz, *A rch. f. Zellforsch.*, VIII., 1912.

The principal stages in the spermatocytic development of *Simocephalus* are indicated in Fig. 3. A resting spermatogonium is shown in Fig. 3, *a*. The spermatogonial chromosomes appear to be slender more or less U-shaped rods. During metaphase (Fig. 3, *b*), they are too closely massed to be counted. I have no doubt, however, that they are considerably more than eight in number. Nuclei in synizesis (Fig. 3, *c*) show a decided contraction of the chromatin threads. No growth occurs during this stage and no nucleolus is discernible. In the prophase of the primary spermatocyte (Fig. 3, *d*), eight distinctly double rod-like chromosomes are evident. The chromosomes in this stage are very distinct being more or less regularly distributed just under the nuclear membrane and in over fifty cases counted the double chromosomes were constantly eight in number.

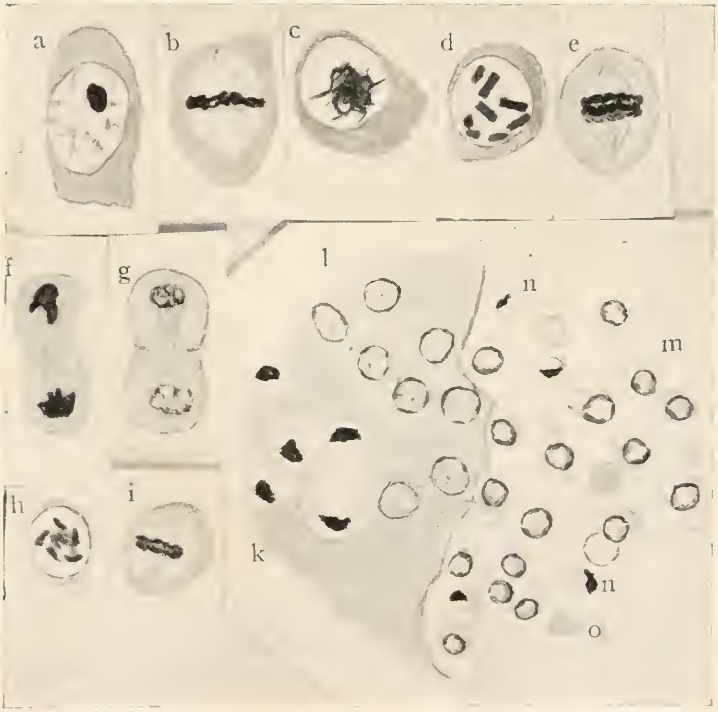

Fig. 3.

Fig. 3, *e* and *f*, show the primary spermatocyte in metaphase and telophase. The chromosomes then pass into an interkinetic resting nucleus, Fig. 3, *g*. The resolution of the chromosomes of the two daughter cells into a resting nucleus is not always synchronous. One may often find an interkinetic nucleus at one end of the telophasic spindle while the chromosomes at the other end are still massed in a densely staining body. There is no doubt, however, that both ends pass into the resting state and form normal nuclei for one may find the entire contents of an island in interkinesis. And in still older islands all the cells pass into Metaphase II., leaving no cells behind. Fig. 3, *h*, represents the nucleus of a secondary spermatocyte in prophase. The chromosomes are approximately eight in number. They are shown in Fig. 3, *i*, in metaphase. Fig. 3, *k–o*, show the cellular elements lying in part of the lumen of a maturing testis and a portion of the adjacent wall. The secondary spermatocytes, Fig. 3, *k*, are shown in telophase. At *l* are spermatids with vesicular nuclei. Their arrangement in islands is better shown in the upper part of Fig. 2. Upon the disintegration of the surrounding cytoplasm, the spermatids come to lie in the lumen of the testis. Here, Fig. 3, *m*, the nuclei contract somewhat and become more densely chromatic.

In some of the spermatids, Fig. 3, *n*, *n*, the contents of the nucleus collects into a compact eccentric mass, which finally disintegrates and disappears.

In other spermatids the nucleus remains vesicular and it is this second type only that is to be found in the distal end of the vas deferens of a mature testis.

That approximately half of the spermatids degenerate is the impression gained by the examination of sagittal sections of entire testes.

Lepeschkin,[1] in a brief paper in Russian, kindly translated for me by Dr. M. Scholtz, of Cincinnati, on the spermatogenesis of the Daphnid, *Moina rectirostris*, describes occasional degenerating cells not only among spermatids but also among spermatocytes and spermatogonia. He speaks of the uniformity of the cellular elements in young testes and of the formation of cysts enclosing spermatocytes, all the contents of a cyst being in the same stage of development. He does not describe nuclear changes, giving, as an excuse, the diminutive size of the cellular elements. His figures, showing degenerating cells, are not conclusive for one might easily confuse synizetic and interkinetic nuclei and possibly different stages of the giant cells with degenerative appearances. Occasional abnormalities do occur in any gonad but in healthy normal specimens studied by me I have been unable to discover degenerative appearances except among spermatids.

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[1] W. D. Lepeschkin, *Mem. Soc. Amis Sc. Nat. Anthrop. Ethnogr. Univ. Moscou*, Vol. 98, Sect. Zoöl., Vol. 3, No. 9, 1907.

Two classes of spermatids, of which one only produces functional spermatozoa, are described by McClendon[1] in his studies in the spermatogenesis of *Pandarus sinuatus*, a parasitic Copepod, a species which exists only in the sexual state. According to McClendon some of the spermatids are transformed into "nutritive spheres." The "spheres" are a constant feature in *Pandarus* and the proportion formed is very large. On the assumption that spermatids occur in male- and female-producing classes, this condition might possibly disturb the sex ratio of the species. This is not true for I have collected large numbers and have always found the males and females in approximately equal numbers.

A personal study[2] of the spermatogenesis of *Pandarus sinuatus* has convinced me that McClendon's "nutritive spheres" are derived not from spermatids but from spermatocytes during interkinesis. If, then, we assume post reduction for the sex-determining factor, a condition which obtains in most forms where a distinct accessory or "sex" chromosome occurs, the formation of the "nutritive spheres" will be from neutral cells and should, therefore, cause no disturbance in the ratio of male- and female-producing sperm.

In Aphids[3] two classes of sperm are produced in the male differing in the presence and absence of an accessory chromosome. The class which does not possess the accessory chromosome degenerates. By inference from other groups of insects this is the male-producing class. The other class produces functional sperm which, entering the egg, give rise to females only. These sperm correspond to the female-producing sperm of other insects.

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[1] J. F. McClendon, *Arch. f. Zellforsch.*, V., 1909.

[2] R. Chambers, Jr. In press.

[3] T. H. Morgan, *Proc. Soc. Exp. Biol. and Med.*, 5, 1908, and *Science*, Vol. 29, 1909. W. von Baehr, *Arch. f. Zellforsch.*, III., 1909.

In *Simocephalus* no accessory chromosome is to be distinguished, the sperm being apparently all alike in their chromosome number.

No means as yet have been found to distinguish two classes. The presence of degenerating sperm in the lumen of the testis does not necessarily prove the existence of two classes of sperm. However, as the functional sperm enter eggs which develop only into females, the assumption is permissible that these are the female-producing sperm and that the male-producing sperm are those which degenerate.

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BIOLOGICAL BULLETIN

LOCAL DISTRIBUTION OF GRASSHOPPERS IN RELATION TO PLANT ASSOCIATIONS.

ARTHUR G. VESTAL.

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I. INTRODUCTION.

During the summer of 1912, while at the biological station of the University of Michigan, at Douglas Lake, the writer found opportunity to study the relation between local distribution of grasshoppers and the plant associations. The group studied includes the three subfamilies *Tryxalinæ*, *Œdipodinæ*, and *Acridiinæ*, of the orthopteran family *Acridiidæ*.

Habitat-distribution of *Orthoptera* has received considerable attention. Published accounts of *Orthoptera* contain the bulk of the data on habitats. The studies of Morse ('04), and of

Hancock ('11), classifying these insects on the basis of habitat, have been reviewed by Shelford ('12*b*: 352). Biological surveys of certain regions, as the Michigan surveys, have included local distribution of grasshoppers (Adams, '06, '08; Ruthven, '11; Hart and Gleason, '07). Studies of animal communities have included data on grasshopper distribution (Shelford, '12*a*; Vestal, '13*b*). In these two studies not all of the associations of the region were considered. In many of the above accounts the concept of the plant association as a habitat has appeared incidentally or not at all; this concept was used in a study of local distribution of birds, by Gates ('11). In the present study all the plant associations of the region have been included, and the plant association has been used as the index of the habitat. In general the results indicate that the important factors of local distribution are, in initial stages of development of the vegetation, *physical* conditions of the environment; in advanced stages, *vegetational* conditions; in either case, the character of the plant association is the index to local environmental conditions for grasshoppers. Collection data are in another paper (Vestal, '13*a*).

Physiography of the Region.

Douglas Lake is situated less than twenty miles south of the northern tip of the southern peninsula of Michigan, in Cheboygan county. There are two main physiographic types, the first being old beaches and lake bottoms of the Nippissing and Algonquin Lakes, and the second morainic in origin. The soil of the first type is almost pure sand; the relief is very slight. The morainic lands have typical rolling topography; they are of mixed composition, usually with loam or sandy loam surface soil. Streams are small and few, but on the whole the region is well drained, the soil being porous. Swamps occupy a very small proportion of the area, and are usually drained. Undrained swamps are few and small in the immediate vicinity.

The lake is about four miles long, with irregular contour, and lies north of a chain of lakes which in recent geological time connected Lakes Michigan and Huron. Douglas Lake was itself, previous to this time, a part of the connection. Much of the shore line is sandy beach, with occasional sand-bars and spits, and in several places lagoons. Wave action is considerable on exposed parts of the shore-line, but the exposed sand has been very little acted upon by wind. Beach dunes occur sparingly and are of small area.

General Character of the Vegetation.

Much of this part of Michigan is covered with two general types of vegetation, which give rise to the common terms "pine lands" and "hardwood lands." The pine forests have been developed on the more sandy areas, the hardwoods on the loamy or clay moraines. "Hardwood lands" are of much greater agricultural value, and the more extensive farming districts are in morainic regions. The pine lands have been largely deforested, and fire has also been very prevalent, so that many of the original pine forests have been replaced by growths of the large-toothed aspen. In the immediate region of the biological station virtually no pine forest remains, though scattering growths of pitch pine are not rare along the beach, and a few pines are to be found scattered among the aspens. The aspen forest occupies more than one half of the entire area studied; the hardwood forest and hardwood clearings somewhat more than a fourth of the area, and other kinds of vegetation considerably less than one fourth. These other kinds of vegetation are grassland areas, cultivated fields, meadows and sedge growths, cedar bogs, and open peat bogs. The plant associations have been studied in detail by Gates ('13), with reference to a much larger area than has been covered in the present study.

II. The Plant Associations and the Grasshoppers which Occur within Them.

Associations of two vegetation regions or *vegetation provinces* (Gleason, '10: 42–45) occur within the area. The coniferous forests, the aspen forests, heaths and bogs of the region, typical of the northeastern states and much of Canada, represent the *Northeastern Conifer Province.* The deciduous forest (hardwoods) and the herbaceous and thicket growths of hardwood clearings represent the *Eastern Deciduous Forest Province.* While primarily consisting of these two geographic elements, the vegetation also includes *local associations,* particularly those determined by local conditions of moisture; these are usually not characteristic of any one vegetation province; and *ruderal associations*, composed of introduced plants. Plant names used are those of Gray's Manual, seventh edition.

Associations of the Northeastern Conifer Province.

The culminating type of vegetation in the Northeastern Province is the balsam-spruce-birch forest, which is developed successively through lichen, heath, and different pine stages, in xerophytic situations; and through bog and bog-forest stages in water and wet ground. Only those associations are listed which are represented by distinct areas in the region studied.

Chamædaphne Association (Gates, '13: 57).—No grasshoppers were taken in the open bogs of cassandra. In Ontonagon county, northern Michigan, thickets of cassandra, alder, wax myrtle, high-bush cranberry, etc., are the habitat for *Podisma glacialis* and *Melanoplus islandicus* (Morse, '06: 70).

Thuja Association (Gates, '13: 66).—The cedar or arborvitæ growth known as Rees's bog, near the shore of Burt Lake, south of Douglas Lake, was studied. In this bog the peat soil is less than two feet deep, the substratum being sand or gravel. It is drained through the porous soil into Burt Lake. No bare soil is exposed, but partly moss-grown logs lying upon the surface are not infrequent. Trees are usually not more than twenty feet in height, but are close together, and cast a deep shade. *Thuja occidentalis* is the dominant species; *Larix laricina*, tamarack, and *Picea mariana*, black spruce, occur sparingly. The surface is a thick carpet of *Sphagnum;* many peat-bog plants, as *Cornus canadensis*, orchids, ericads, etc., are present, more abundantly in less shaded parts. The only grasshopper species taken in this bog is *Melanoplus islandicus*.

Aspen Association (Gates, '13: 77).—The extensive sandy pine lands of the region are now, as a result of fires and cutting, practically all occupied by the aspen forest. The dominant tree is the large-toothed aspen, *Populus grandidentata*. Other frequent species are paper birch, beech, red oak, hard maple, red maple, wild cherry, *Prunus pennsylvanica*, white pine, pitch pine. The undergrowth, quite similar to that of the pine forest, is chiefly composed of bushy blueberry plants, *Vaccinium penn-*

sylvanicum, which are close together and usually less than six inches high, and of the taller and less numerous plants of the bracken fern, *Pteris aquilina*. Other plants are the bush honeysuckle, *Diervilla lonicera*, and several grasses and composites. Near the lake beach patches of bearberry, *Arctostaphylos uva-ursi*, and of dwarf juniper, *Juniperus horizontalis*, form small heaths. Over all the open aspen growth, a considerable proportion of bare sandy soil is exposed, in the interspaces between plants. Dead leaves, partly decayed and partly burned stumps and logs, litter the surface. There are a number of bare roadways.

The tree growth is irregular, being entirely absent in frequent local areas which vary in size from small unshaded patches between trees to areas thirty meters in diameter. In such places there are indications that the undergrowth is practically independent of the trees. In the older tree growths the hardwood species have assumed control, indicating development into hardwood forest. Ground conditions are more like those of closed forest.

In the treeless parts of the association, in the bracken-blueberry growth, *Melanoplus angustipennis* is the common grasshopper species. *Melanoplus atlanis* and *Camnula pelludica* are common, and *M. bivittatus* occasional, along roadways. *M. luridus* is found sparingly in scattered aspen growths. *Scirtetica marmorata* occurs usually on or near the lichen-covered surfaces. *M. fasciatus* is more often found in the closed forest. On the sandy roads, and sparingly in the sandy interspaces between the plants, are found *M. atlanis*, *M. angustipennis*, *Dissosteira carolina*, *Spharagemon bolli*, *Circotettix verruculatus*, *Arphia pseudonietana*, *Hippiscus tuberculatus*.

Associations of the Eastern Deciduous Forest Province.

The most highly developed form of deciduous forest vegetation is the beech-maple or beech forest, well represented in the region. This develops, on dry soil, through herbaceous, thicket, and xerophytic oak stages, usually, followed by mesophytic red oak and maple stages. The following associations were studied in the Douglas Lake region:

Herbaceous Associations (Gates, '13: 75).—The common fireweed, *Epilobium angustifolium*, is the first plant to establish itself on newly cleared or burned hardwood land. The clearings are soon overgrown with herbaceous plants, many of them introduced, as mullein, *Verbascum thapsus*. These burns and clearings vary considerably both as regards physical conditions and plant composition. They are only temporary stages in the process of reforestation. Usually they are dry and hot, being fully exposed to the sun, but often sheltered from wind by surrounding forests. Grasshoppers are numerous, both in individuals and in species. In approximate order of abundance they are: *Melanoplus atlanis*, *Camnula pellucida*, *Dissosteira carolina*, *M. bivittatus*, *Spharagemon bolli*, *Circotettix verruculatus*, *M. luridus*, *Chloealtis conspersa*, *M. minor*. Only the first two occur in any considerable abundance.

Thicket and Bramble Associations (Gates, '13: 76).—The shrubby plants which replace the herbs in clearings are principally blackberry and raspberry, *Rubus* spp., red-berried elder, *Sambucus racemosa*, and young seedlings and shrubs of hard maple, *Acer saccharum*. These growths often form a dense tangle which is almost impenetrable. In dense parts of these thickets an occasional *Melanoplus bivittatus* would be seen upon a leaf at the top of a shrub. Where the ground could be seen, *Melanoplus atlanis*, *Camnula pellucida*, and *Chloealtis conspersa* were also to be found.

Beech-Maple Association (Gates, '13: 71).—The beech-maple forest is dominated by two tree species, *Fagus grandifolia*, which occurs in places in nearly pure stands, and *Acer saccharum*. *Tsuga canadensis*, the hemlock, is also important in places. *Ostrya virginiana*, *Betula lutea*, and *Tilia americana* are infrequent species. In the deeply shaded parts of the forest, the undergrowth consists mainly of young seedlings of *Fagus* and *Acer*, with small plants of *Maianthemum canadense* and *Mitchella repens*, partly hidden in the thick carpet of fallen leaves. In the sunlit spots the undergrowth is taller, with *Acer pennsylvanicum* and *Sambucus racemosa*. Many other forest plants occur. Stumps and logs are common, but no bare ground is exposed. The relative humidity is high. Exposure to sun and wind is at a minimum. *Melanoplus islandicus* is a characteristic grasshopper species, of the deeply shaded parts of the forest. It is probable that *Podisma glacialis variegata* occurs also on shrubbery. In more open parts of the hardwood forest, and near the edges of clearings, most of the grasshopper species that are common in clearings were also found, in very small numbers, however.

Ravine Forest Association (Gates, '13: 66–70).—This association is best developed in the ravine occupied by what is locally known as *Big Springs*. A number of springs, fed by the underground drainage from Douglas Lake, to the north, form the head of Carp Creek, which runs south to Burt Lake. The ground is very wet, and is occupied by bog plants and deciduous forest undergrowth. Trees are *Tilia americana*, *Tsuga canadensis*, *Fagus grandifolia*, *Ostrya virginiana*, *Betula lutea*, *Acer saccharum*, *Abies balsamea*. Succession has proceeded from the coniferous bog forest toward the beech-maple association. *Podisma glacialis variegata*, recorded from Carp Creek, probably was taken in this ravine forest, *Melanoplus islandicus* probably also occurs in the same association.

Local Associations.

Dry Beach Associations (Gates, '13: 55).—Quite a number of the strand plants of the sandy margins of Douglas Lake belong to an assemblage which is characteristic of the sand shores of the Great Lakes. Three of these independently form associations in the region. These are *Elymus canadensis*, *Ammophila arenaria*, and *Potentilla anserina*. *Elymus* forms a low, narrow dune about one eighth mile long, at the southeastern end of the lake. It and *Ammophila* are found just above the level of wave action at many places on the shore. Sandbars on the north shore were partly covered by *Potentilla* growth. In these beach associations the soil is pure sand, dry and shifting, with full exposure to sun and wind. The vegetation is dry and scanty. Grasshoppers typical of these situations are: *Melanoplus atlanis*, *M. angustipennis*, *M. bivittatus*, *Camnula pellucida*, and *Dissosteira carolina*. Two species not taken at Douglas Lake, *Spharagemon wyomingianum* (Thomas), and *Trimerotropis maritima* (Harris), are very characteristic of beaches and dunes of the Great Lakes; both are recorded by Shull from the Saginaw Bay region ('11: 225–226).

Marsh Associations (Gates, '13).—Communities of marsh plants bordering lakes, streams, and certain open bogs are usually composed of only one or a few plant species. These growths, depending upon local conditions of moisture, are frequently small in area, and different stations are not always alike. Conditions of shade and of soil are variable. No grasshoppers were found upon the marsh plants which grew standing in open water, although in dense growths individuals sometimes stray beyond the shore. The numbers of grasshoppers vary considerably in different stations. They are most numerous in tall, rather close, sedge or grass growths. *Stenobothrus curtipennis* is the characteristic grasshopper of littoral situations. *Melanoplus atlanis*, *M. bivittatus*, and *M. differentialis* are less frequently found. *M. femur-rubrum*, though not taken by the writer, is often found in such places. The *Melanopli* are more numerous in the higher and more open parts, while *Stenobothrus* occurs farthest out towards the water.

Ruderal Associations.

In the Douglas Lake region much of the native forest has been removed by cutting and burning, and its place has been taken by cultivated crops and weed growths. In addition many plant associations, though not destroyed, have been materially changed, and native animals have also been much affected. Secondary successions are principally due to interference by man. Most of the sandy land is now occupied by aspens as a result of destruction of the pines. One of the most important plants favored by artificial conditions is the blue-grass, *Poa pratensis*, which enters into nearly all ruderal growths. Other species of *Poa* probably occur in the region.

Ruderal Dry Grassland Associations.—Abandoned fields, dry pastures, roadside growths, and modified aspen undergrowth are the common forms of ruderal growth in dry situations. They are necessarily very different in physical conditions and plant composition, depending upon differences in original status and subsequent modification. In certain large areas west of Douglas Lake, near Pellston, a growth of bluegrass has almost entirely replaced the aspen association. Near the lake bluegrass invades the aspens along roadways, and one can trace long abandoned roads by the presence of this plant. Other weed species are numerous. Grasshopper species of ruderal dry grassland are, in approximate descending order of importance: *Melanoplus atlanis*, *Camnula pelludica*, *M. bivittatus*, *Dissosteira carolina*, *Arphia pseudonietana*. All of these species, with the possible exception of the last, are more abundant in ruderal than in native vegetation.

Sparsely Vegetated or Bare Soil.—The condition of bare soil is much more frequent, in humid climates, as a result of disturbance. Plowed fields and constantly trampled paths and roads are the commonest areas of bare soil, and these furnish suitable habitats for those species which normally rest on bare soil, not on the plants. The *Œdipodinæ* are of this habit to a large extent. The character of the soil is important for oviposition, and though the bare soil grasshoppers are more or less migratory, certain of them are found associated with particular types of soil. Hart gives the soil-preferences for a number of the *Melanopli* in a sand region in Illinois ('07: 214, 215). It is to be remembered that bare soil as a habitat is not sufficient; nearby vegetation is necessary, and in this region is always present. Where extensive areas of bare soil occur, grasshoppers are very rare except at the border. The insects are conspicuous in bare places, as roads, but are more abundant in the interspaces between plants, in open growths. Areas of bare soil differ from the sparsely vegetated areas merely in degree, and are really the same kind of habitat. In the region studied considerable bare soil is exposed along the beach, but in the aspen association roads are the typical areas of bare and sparsely-grown soil, as also in the hardwood district. Interstitial grasshoppers which are conspicuous in bare soil are: *Dissosteira carolina*, *Arphia pseudonietana*, *Circotettix verruculatus*, *Spharagemon bolli*, and *Hippiscus tuberculatus*. Those of open grassland, frequently found on bare soil of interstices, and less frequently on bare soil of roads, are: *Camnula pellucida*, *Melanoplus atlanis*, *M. bivittatus*, *M. angustipennis*.

Meadow Associations.—Ruderal meadows and swales, like the native marsh and littoral associations, are variable in character. Probably the most common type is the bluegrass-white clover meadow. It is found in wet pastures and along boggy roads; it forms a very low, dense carpet, resembling a closely trimmed lawn, and probably always depends on constant cropping of cattle. It is of common occurrence on the north shore of Douglas Lake, and near Munro Lake, several miles north. In this type of meadow *Melanoplus atlanis* and *Stenobothrus curtipennis* are of about equal abundance. *M. femur-rubrum* was not taken, but is more abundant in such places in other localities than is *M. atlanis*. *M. differentialis* is typical in meadow habitats, and *M. bivittatus* is found sparingly.

Arrangement of the Associations as Habitats.

The associations of any particular region may be placed in groups with respect to several well-known criteria. One of the prevalent methods is to consider together all associations which are genetically related, which form a developmental series, each stage being succeeded by the next in order, until the ultimate or climax association is reached. Another mode of classification is geographic, placing together all associations of like geographic distribution, all typical of a definite climatic region, an ecological province. Another grouping is based upon growth-form: by this treatment associations dominated by plants of similar growth-form, indicating physiological likeness, would be considered similar, regardless of geographic or successional relationships. The common division of associations of a region into climatic or geographic, and edaphic or local, is based upon local distribution or habitat. The grouping into aquatic and terrestrial is a grouping based upon habitat in a larger sense: the medium of life is the important feature. Finally it is necessary to distinguish between primary, original or native associations, and secondary, ruderal or cultural associations, wherever human influence has modified primeval conditions. These different groupings are not at all parallel, but form a complex. To orient an association with respect to others of the same region it is necessary to find its place in this complex. In a regional treatment it is usually necessary to consider all of these criteria. For different purposes, different bases of classification may be emphasized, but in no case should one basis be confused with another.

For the purpose of the present study, in which the plant associations are considered mainly as habitats for grasshoppers, two bases of classification receive emphasis. The first is with respect to habitat, the second with respect to growth-form of the plants. These two considerations determine the physical and vegetational conditions to which the grasshopper species are subjected.

It is to be understood that grasshoppers are animals of the ground stratum and of the herbaceous stratum. In so far as these strata are similar as habitats in different plant associations, these associations may be treated together. Considerations as to whether associations are proximate or ultimate, geographic or local, native or ruderal, whether they belong to the Northeastern Province or the Deciduous Forest Province, are of interest only as they affect relative area of different associations within the region, and consequently the relative frequency and abundance of the grasshopper species of the different associations. In the following synopsis, the moisture factor is used for the division according to habitat, though other physical factors, such as soil, might have been used if it had been desirable to subdivide further. The synopsis arranges the associations of the region according to their similarity as habitats for grasshoppers.

- Herbaceous or grassland associations.
  - In wet or moist habitats.
    - Sedge and other littoral associations.
    - Meadow associations.
  - In dry habitats.
    - Sparsely vegetated or bare soil.
    - Beach-grass associations.
    - Ruderal dry grassland.
    - Hardwood clearings.
    - Bracken-blueberry growth of open aspen forest.
- Forest associations.
  - In wet or moist habitats.
    - Cedar bog forest.
    - Ravine forest.
  - In mesophytic habitats.
    - Closed aspen forest.
    - Beech-maple (hardwood) forest.

Among the dry grassland associations, variable conditions (resultants of both physical and vegetational agencies) are: texture and compactness of soil, humus content, proportion of bare surface; height, density, and general character of the vegetation. Sparsely vegetated grassland has a high proportion of bare surface; roadways and other small areas of bare soil are to be considered as part of an area of open vegetation.

It should be pointed out that conditions in the ground stratum of the aspen association are the same in the open treeless parts as among scattered trees. Exposure to sun and wind, as well as the vegetation, are almost identical in the two places. The open aspen forest is then, so far as its ground stratum is concerned, *a grassland association*. This is in accord with the results of Shelford ('12*a*: 82), who found that ground stratum conditions in open forests in sand at the lower end of Lake Michigan lag behind in the succession from herbaceous to forest growth. In reality open forests of very scattered trees are usually mixed associations, dominated in places by trees, and in more open places by herbaceous plants. So far as ground conditions are concerned, the association is herbaceous, and the bracken-blueberry growth is accordingly grouped with dry grassland. Ground conditions are not those of forest until the closed stage of the aspen association is reached. The lichen growth of open aspen areas, affording a locally different environment, may be regarded as a minor division of that association.

A primary division in the above synopsis, "shrub associations," might have been added. The thicket and bramble associations were small in area, were little studied, and as the conditions in the ground stratum in thickets are much the same as those in the hardwood forests, with about the same grasshopper assemblage, this division is omitted for simplification.

The grasshopper assemblages of these associations are presented in summarized form in the third section of this paper, which here follows.

III. SUMMARY OF LOCAL DISTRIBUTION OF THE SPECIES.

The table which follows summarizes the habitat relations of the grasshopper species, and includes an approximate estimate of the numerical status of each species in each of its habitats. The method of numerical estimation is described on p. 165. It is necessary at this point to define the terms used to denote degrees of frequency, or regularity of occurrence, and abundance, or numbers of individuals per unit of area. *Dominant* species in a habitat, usually only one or two, have a very high abundance, and a high frequency, in that habitat. *Abundant* species are also

TABLE I.

SUMMARIZING HABITAT RELATIONS OF THE GRASSHOPPERS OF THE DOUGLAS LAKE REGION.

Terms denoting degrees of frequency and abundance are defined immediately above, and are abbreviated in the table. Question-marks are inserted to indicate probable occurrence in habitats little studied. Where a space is blank it is understood that the species occurs accidentally or not at all in the particular habitat.

	Moist Grassland.		Dry Grassland.					Forest.			
	Sedge Growths.[2]	Wet Meadow.[2]	Bare Soil.[2]	Beach-grass.[2]	Ruderal Grass-land.[2]	Bracken-Blueberry.	Hard-wood Clearings.	Closed Aspens.	Beech-Maple.	Ravine Forest.	Thuja Bog.
Stenobothrus curtipennis (Harris)	Freq.	Freq.		Accid.							
Melanoplus differentialis (Thomas)		Freq.			Freq.	Occas.	?		?		
Melanoplus bivittatus (Say)		Freq.	Infreq.	Occas.	Freq.[1]	Occas.	Freq.		Infreq. or Accid.		
Melanoplus femur-rubrum (De Geer)		Freq.?		?	Freq.?	?	Freq.?				
Melanoplus atlanis (Riley)		Abund.	Freq.[1]	Abund.	Dom.	Freq.	Abund.	?	Infreq. or Accid.		
Camnula pellucida (Scudder)		Accid.	Freq.[1]	Freq.[1]	Dom.	Freq.[1]	Abund.	?	Infreq. or Accid.		
Melanoplus minor (Scudder)							Infreq. or Occas.				
Melanoplus luridus (Dodge)				?	?	Occas.	Occas.				
Melanoplus angustipennis (Dodge)			Occas.	Freq.	Infreq.	Dom.					
Dissosteira carolina (Linnæus)			Freq.[1]	Freq.	Freq.[1]	Occas.	Freq.[1]				
Spharagemon bolli Scudder			Freq.[1]	Occas.		Occas.	Occas.		Infreq. or Accid.		
Arphia pseudonietana (Thomas)			Freq.[1]	Infreq.	Freq.[1]	Infreq.	?				
Hippiscus tuberculatus (Palisot)			Freq.	Infreq.	?	Infreq.	?				
Circotettix verruculatus (Kirby)			Freq.	Occas.	Occas.	Occas.	Occas.		Infreq. or Accid.		
Scirtetica marmorata (Harris)						Infreq.[3]	?	?			
Melanoplus fasciatus (Barnston-Walker)						Infreq.	Infreq.[4]	Freq.[1]			
Chloealtis conspersa (Harris)						?	Occas.	Infreq. or Occas.	?		
Podisma glacialis variegata Scudder									?	Occas.?	?
Melanoplus islandicus Blatchley									Freq.	?	Occas.

[1] Frequently found, usually not in numbers, but in a few places approaching abundance.

[2] Stations representing these five habitats were rather variable as to physical and vegetational conditions, and also as to the numbers and species of the grasshoppers. Data for these five columns were compiled from more detailed tables in which a vertical column was assigned to each station of each of these habitats.

[3] More typical of the lichen-covered surface in more or less sheltered parts of the bracken-blueberry growth. In the association as a whole the species is infrequent; in lichen patches it is frequent.

[4] One specimen of the long-winged form, *M. f. volaticus*, was taken in the grassy border of a growth of hardwoods.

frequent; they are usually to be found in the habitat in considerable numbers at any time. *Frequent* refers to grasshoppers occurring regularly in the habitat, though not always to be found, and seldom numerous when found. *Occasional*, occasionally found in the habitat, not frequent or abundant. *Infrequent*, not often found in the habitat. *Accidental*, occurring in a habitat rare or unusual for the species. Specimens are usually found singly and the abnormal habitat frequently adjoins one in which the species is more regularly found.

The obvious facts shown by the table are:

1. Grasshoppers are more abundant, in species and in individuals, in herbaceous or grassland habitats than in forest, and more abundant in dry than in moist or wet situations.

2. Certain species are much more restricted than others in range of habitats, and in accompanying range of toleration of physical and vegetational factors of the environment.

3. Although a species may be found over several associations, it is more abundant in one, or two, of these, than in others (Certain activities take place in more restricted habitats; chief of these restricted activities is the laying of eggs.)

4. No two plant associations have identical grasshopper assemblages.

5. No two grasshopper species have identical habit-preferences.

These facts and others are considered in order in the general discussion.

In general, with every change of habitat there is a change in the assemblage of grasshopper species. These replace one another in the various habitats, often with considerable overlap, but all can be arranged with respect to gradients of environmental conditions. Gradients of several factors which vary together may in general be said to run parallel with the development of vegetation. This development of vegetation is in part the result, in part the cause, of these changes of physical and vegetational conditions.

The variation of environmental conditions can be expressed graphically by lines representing gradients of the factors which change with development of vegetation. Environmental conditions in each successional series may be represented by a line, at one end of which the conditions are those of sterile soil or water, environmental control being entirely physical; at the other end conditions are those of the closed, completely developed, climatic association, vegetational control of local environment being nearly complete.

It is further possible to arrange the lines representing the different successional series within a region, so that relations between them may also be seen graphically. Fig. 1 (p. 156) is an arrangement of the gradients of physical and vegetational conditions in the Douglas Lake region, based on the development of vegetation, and accompanied by a representation of the selection of these conditions by the various grasshopper species.

IV. General Discussion.

The Assignment of Terrestrial Animals to Habitats.

It is thought by some zoölogists that the local distribution of most terrestrial animals is more or less haphazard, that there is no order in the distribution of animals into different habitats, or that, if there is order, the conditions of distribution are too complicated to be determined by any present methods. Shelford ('11: 591) points out several reasons for the prevalence of these and similar opinions. On the contrary, environmental relations of animals are now coming to be recognized as quite definite (Shelford, '12*b*: 333).

The habitat, in the sense of abode for animals, is a particular combination of certain environmental conditions, physical and vegetational, and to some extent, animal, uniform over a certain area. More or less variability within this area is the rule, and we may consider the area of the habitat as larger or smaller, according to the degree of uniformity of conditions. In these areas of varying degree, plant and animal communities of various degree find their existence. Ecological classification, aside from its dealings with plant and animal individuals, has to do with the recognition and classification of these degrees of likeness and difference of environments and of plant and animal communities. (See Shelford, '12*b*: 355.) The habitat considered most convenient in the treatment of local distribution of

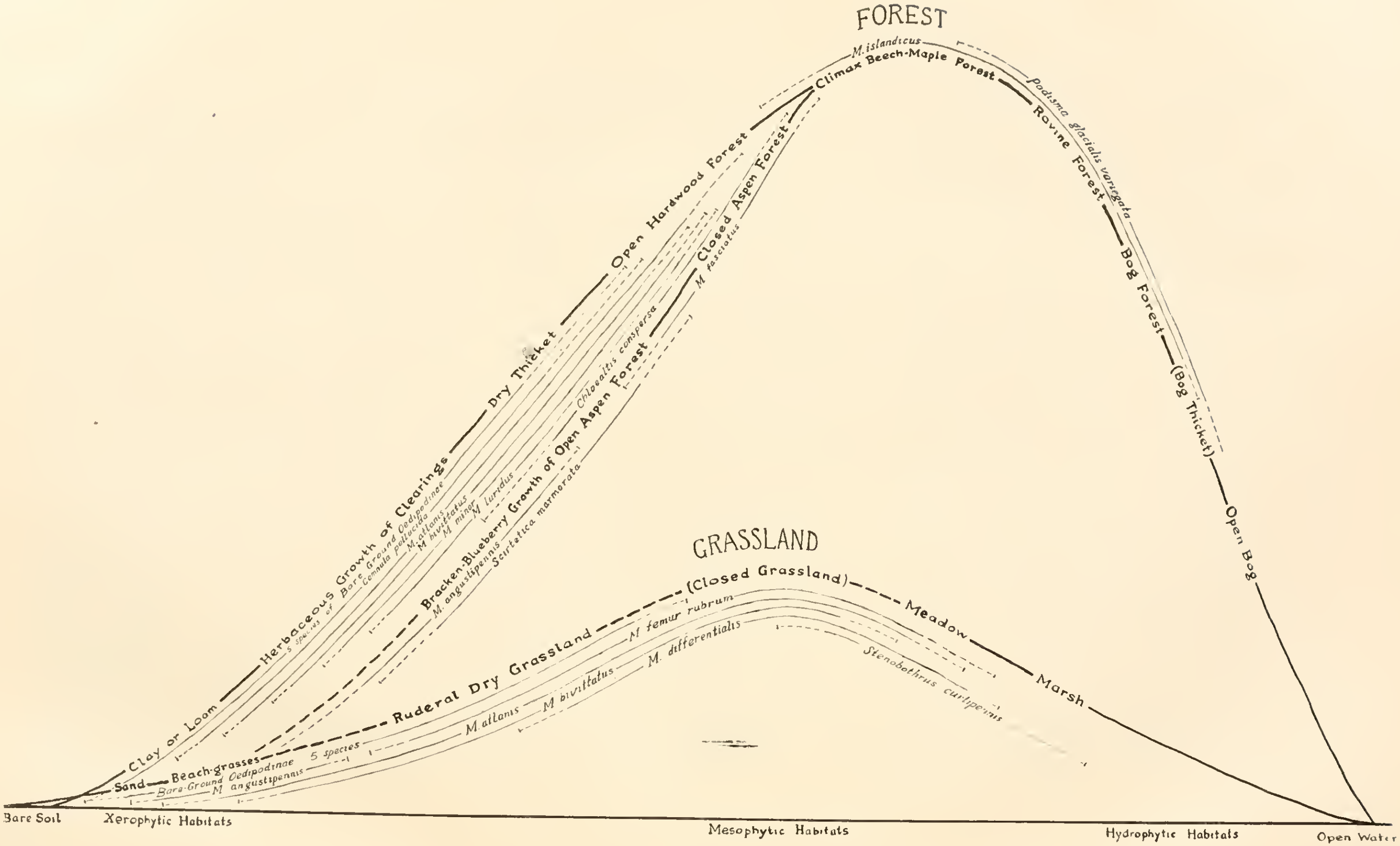
FOREST
M. islandicus
Climax Beech-Maple Forest
Podisma glacialis variegata
Ravine Forest
Bog Forest
(Bog Thicket)
Open Bog
Open Hardwood Forest
Closed Aspen Forest
M fasciatus
Dry Thicket
Chloealtis conspersa
Bracken-Blueberry Growth of Open Aspen Forest
M. angustipennis
Scirtetica marmorata
Herbaceous Growth of Clearings
5 species of Bare Ground Oedipodinae
Camnula pellucida
M. atlanis
M bivittatus
M minor
M luridus
Clay or Loam
Sand
Beach-grasses
Bare-Ground Oedipodinae
M angustipennis
5 species
GRASSLAND
(Closed Grassland)
Meadow
Marsh
Ruderal Dry Grassland
M femur rubrum
M. differentialis
M bivittatus
M. atlanis
Stenobothrus curtipennis
Bare Soil
Xerophytic Habitats
Mesophytic Habitats
Hydrophytic Habitats
Open Water

FIG. 1. Showing the relation between grasshopper species and gradients of physical and vegetational conditions within the Douglas Lake region. At the left are initial stages of vegetation developing from dry ground; at the right initial stages developing from wet ground or open water. Conditions vary as development of the vegetation proceeds; at the top of the curve xerophytic and hydrophytic series converge in the climax association. Vegetational control is, roughly, successively greater from bottom to top. Moisture content is successively higher from left to right. Only the initial stages of grassland vegetation would normally be represented in the region, as forest invasion would prevent development of closed grassland. However, certain ruderal associations within the region present conditions essentially those of partially and of nearly closed grassland, and these associations are accordingly placed in the proper place in the gradient of grassland environmental conditions. Stages not well represented within the region are in parentheses (bog thicket, closed grassland). In certain of the gradients the transition between two stages is not direct, as between beach-grass and open aspen forest. Such indirect transitions are represented by dotted lines. The gradient for development of coniferous forest is not shown; it might be represented by a line parallel to the other dry forest gradients.

Approximate range of the grasshopper species in the various gradients is shown by fine lines, which are dotted in the extremes of the range, in which their occurrence is infrequent and their numbers small. It is seen that certain species occur in equivalent stages of the different kinds of vegetation. Species common to hardwood and aspen forests are placed between the two gradients; those restricted to aspen forest are to the right of the aspen gradient.

grasshoppers within a region is the area marked off by the plant association, in which there is a general and usually recognizable uniformity of vegetation and of physical conditions.

A species is to be assigned to a habitat if its occurrence in that habitat is a matter of regularity. In general, the animal species is seldom confined to a single habitat within a region. The determination of the more typical habitats selected by a particular species involves estimation of relative numbers, and presents certain difficulties, which are discussed below. In a regional study of a group of animals taxonomically related, or of all the animals of the region, there are certain other difficulties which obscure the definiteness of habitat relations.

Variation in Range of Habitats in Different Species.—The range of toleration of environmental conditions varies widely among different species of a taxonomic group, and accordingly we find certain animals restricted to one or several similar habitats, while others range over many and different habitats with apparently little discrimination. *Melanoplus atlanis*, for example, occurs regularly in meadows, in dry open grassland, and in open forest. It is a species which can endure widely varying conditions. Upon examination, it is found that it is very much more numerous in certain habitats than in others, and that there are certain habitats in which it occurs only accidentally or not at all. In the Douglas Lake region, it was never seen in cassandra bogs, in bog or ravine forests, in closed aspen forest, and only infrequently or accidentally in closed hardwood forest. There is, then, a *selection* of habitats, and the local distribution of even the most generally distributed forms is far from indiscriminate.

Habitat-Distribution as Affected by Motility of Animals.—The habitat relations of plants are more evident than those of animals because the plant individuals are non-motile, while animal individuals can move about, and it is not to be supposed that they will necessarily stay within a single area of uniform conditions. They continually stray beyond the limits of conditions necessary for all or part of their activities. Among the grasshoppers, certain are much more motile than others, particularly the long-winged and active *Œdipodinæ*. These grasshoppers at times fly high and to considerable distances, and may be seen to traverse areas of dry grassland, of marsh, and of forest. However, while at rest, very few will be found on trees, for example, or in deep shade, or in dense herbaceous vegetation. They will usually be seen in open dry vegetation or on bare soil. If found in other conditions, these will usually be not far distant from such open situations. Stray individuals are not common and are usually found singly. It is not surprising that stray individuals should be more frequent among species which are very abundant in the region, as *Camnula pellucida*, and among species of the more extensive habitats. The motility of animals is perhaps commonly overestimated as a factor in width of local distribution. The daily itinerary of an animal is likely to be more circumscribed than is usually thought. In the case of the grasshoppers again, for most species the *ordinary* mode of progression is walking or crawling, rather than jumping or flying. Usually grasshoppers are noticed only when disturbed, or "flushed." Their behavior, jumping or flying *when disturbed*, is a special reaction to an approaching object. The ordinary activities are much less frequently observed. Birds, our most motile animals, have very definite habitat relations (see Gates, '11). Though the actual number of observed occurrences of stray individuals in unusual situations may be large, these occurrences are very infrequent and exceptional when compared with occurrences in the normal habitat.

Differences of Activity in Different Habitats.—Various activities of the animal may take place in different strata of one habitat, or in different habitats, separated horizontally. The stratum or habitat of greatest importance to the animal is the one in which the most narrowly limited activity takes place, and this activity is usually concerned with breeding (Shelford, '07, '11).[1] In the case of the Douglas Lake grasshoppers, eggs are laid just below the surface of the soil, or at least in the ground stratum, and proper conditions for oviposition are among the most important considerations which determine the presence of the particular species in the region, and *the* important consideration in determining which habitat within the region is most essential to the species. The habitats in which few or no grasshoppers are found are those in which the soil is not suitable for egg-laying.

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[1] On p. 595 (Shelford, '11) are given references to other authors, in which instances of breeding activities as being most narrowly limited are given for birds and for fishes.

In many animals the breeding period occupies a rather small part of the season of activity, and the adult animal may spend but a small proportion of its active life in the breeding stratum or habitat. The habitat in which the species occurs most regularly and in the greatest numbers is the habitat in which the species is of greatest influence. It is the most important habitat of the species so far as plants and other animals are concerned. In associational studies, in which emphasis is laid upon relations between organisms, this habitat is most important. The animal may feed in several habitats, but principally in the habitat in which it is most frequent and abundant. The feeding activities, though of secondary importance (usually) in determining the presence of an animal in a particular habitat and a particular region, are of primary importance in relation to the communities of plants and animals of which the species becomes a member. In the study of all the habitats within a given region, emphasis would be placed on the habitat in which the species is most frequent and most abundant. The success of the species in the various habitats, as indicated by relative numbers, is a measure of the degree of correspondence between the environmental conditions actually furnished within the area of the habitat, and those required by the animal. The habitat in which the species is found most regularly and in the greatest numbers is the habitat in that region which most closely approximates the optimum environment for the species.

The Different Activities in Climatic and in Local Habitats.—It so happens, in probably the majority of the grasshopper species of the Douglas Lake region, that the feeding habitats and the breeding habitats coincide more or less perfectly. In insects of incomplete metamorphosis, as *Orthoptera*, the possibility for the immature animals to correspond in mode of life rather closely to the adults is much greater than in insects in which changes from larva to adult are more radical. Grasshopper nymphs feed and hop about as do the adults. They cannot fly, but as flying more commonly does not take the animal into another habitat, this difference is of minor importance. Where the habitat in which the species is more frequent and abundant is of considerable extent within the region, as in climatic associations, soil conditions, or conditions for egg-laying, would be either uniform throughout the area of the habitat, or else suitable small spots would be scattered about within the area. There would then be no necessity for the females to migrate to a different habitat for oviposition. Thus most of the grasshoppers found in the aspen association would be able to lay eggs within its area, and the nymphs would find food and other necessary conditions in the same place as do the adults. In the beech-maple or cedar-bog forests, which individuals of *Melanoplus islandicus* probably rarely leave, suitable egg-laying sites, in this case wood as found in stumps or logs, are scattered about over the forest floor. It thus appears probable, in the extensive habitats provided in terrestrial climatic plant associations, that the correspondence between the conditions required for breeding and the conditions required for feeding and other ordinary activities, may be quite general. Conditions for egg-laying among insects, for example, may still be more restricted than conditions required for other activities, but the local variability of environmental conditions within the area of the plant association is sufficiently wide, usually, to include the more restricted conditions necessary for egg-laying, or whatever the most narrowly limited activity happens to be. If what is true in the case of grasshoppers is true in a large number of terrestrial animals, as seems likely, this means that the limits of the climatic plant association need not be passed, ordinarily, by a large number of the animal species, since all the necessary conditions are supplied within its area. The animal community of the area may be thus, in large measure, self-contained, and coextensive with the plant community.

In local habitats, on the other hand, which are usually restricted in extent and consequently likely to present less variability of environmental conditions within the area, it is less likely that all the conditions necessary for the animal species will be supplied within the area. The number of animal species which can find all the necessary conditions for existence within the area will be comparatively small; thus many of the species will be obliged to perform certain of their activities in other habitats.

Furthermore, environmental conditions in local habitats are likely to be the extreme, rather than the mean, conditions within the region. Thus sedge marsh habitats in the Douglas Lake region have submerged or very wet soil; in the latter case, the growth may be so dense as to leave no exposed soil. These are extreme conditions for terrestrial habitats. The only grasshopper of the region which was seen in such situations is the ectophytic *Stenobothrus curtipennis*. It is probable that this species deposits its eggs in higher and drier soil. Unlike most of the grasshoppers, its ordinary activities can be carried on in a very humid environment, and the presence of exposed soil is not necessary. The conditions necessary for breeding, however, do not correspond with the extreme condition of wet soil, and must be obtained outside the area of the extreme habitat. The local habitat is less likely to be self-contained than the extensive or climatic habitat.

Difficulties Arising from Habitat Complications.—The habitat relations of animals sometimes are much less evident at the tension zone between two contiguous habitats, and there is the further complication that habitats are sometimes distinguished with difficulty. Boundaries will be invaded by animals of both habitats. The tension zone is much wider, and the confusion greater, between two rather similar habitats. As Shull points out ('11: 221), the determination of habitat relations is difficult in regions where the habitats are small in area and much intermingled. The tension zone presents one great advantage in that it allows determination of which species of one habitat range farthest and most frequently into the adjoining habitat. A graded series of species can be determined, which expresses the resultant of different factors entering into habitat selection of the different species.

Unstable habitats are frequently indicated by the presence of mixed plant associations. These contain representatives from two or more plant communities. Thus near the beach of Douglas Lake, the bracken-blueberry growth is mixed with bearberry and juniper heaths, a few small clumps of beach-grasses, in certain spots, and in others by local growths of ruderal bluegrass. In such mixed areas the animal assemblage is not of the constant character seen in more uniform growths.

Mixed conditions within the area of a habitat also tend to confuse. This is particularly well shown in open forest. *Melanoplus fasciatus* and *Chloealtis conspersa*, of the species studied, are found in open forest with a number of other grasshoppers, but appear to be the only two for which forest conditions are really necessary. All the others are grassland species, and are present because of the grassland environment of the ground stratum. Grassland species may be found in very small sunlit grassy patches within a closed forest. These sunlit patches are not to be considered as part of the closed forest habitat. Probably very few insects of the ground stratum are really typical of open forest.

Roadsides and other local modifications are a source of error in assigning animals to a habitat. A collector traveling along the sandy roads through the bracken-blueberry growth of the aspen forest would see large numbers of bare-ground *Œdipodinæ*, almost all on the roads. He might never realize that these grasshoppers are not particularly common in the undisturbed growth, and that they are animals of the roads and not of the bracken-blueberry growth.

Furthermore, the habitat is not entirely uniform within its area. There are many extremely local environmental differences within the habitat, which influence the animal species as well as the plant species. Thus in scattered parts of the bracken-blueberry growth, occasional patches of lichen-covered surface are found. With these patches is associated the grasshopper *Scirtetica marmorata*. It does not occur over the entire bracken-blueberry growth, as does *Melanoplus angustipennis*, for instance.

Within the habitat there are local differences in the degree of development of the vegetation. This is shown in grassland in the degree of openness of vegetation. Where considerable bare surface is exposed between the plants, bare-soil animals are found. In closed grassland, no animals of this habit occur. Differences of development are shown in the aspen forest. Among scattered trees the bracken-blueberry growth presents an environment essentially that of grassland. In more compact tree growths ground conditions begin to approach those of the forest floor. Only in such situations is the short-winged grasshopper *Melanoplus fasciatus* to be found.

Estimation of Relative Numbers in Different Habitats.

The Distinction Between Frequency and Abundance.—Many collectors do not distinguish between frequency of occurrence and abundance of individuals. To them a species is common if scattered individuals are seen frequently, or if numerous individuals are seen infrequently. Frequency refers to regularity of occurrence in one or more habitats; abundance is concerned with numbers of individuals per unit of area. The distinction is indispensable if we are to estimate numbers of individuals in different habitats. Thus many of the conspicuous *Œdipodinæ*, a number of which can be seen in flight at one time, but over a considerable extent, as far as one can see, are frequent species, but are not numerous as compared with certain inconspicuous *Melanopli*, a larger number of which may be found in any area within the habitat as large as a few meters square. These last are abundant *and* frequent.

Difficulties in the Way of Numerical Estimation.—The writer is not acquainted with any very practicable method of estimating absolute numbers of insects in different habitats of a region. Absolute numbers of plants may be estimated under favorable circumstances, as may also the numbers of the larger or more sedentary animals. Following are some of the difficulties encountered in estimating absolute numbers of terrestrial animals: In any one species, there are differences in numbers of individuals, in degree and kind of activity, in readiness with which it is perceived, in ease with which it is captured, or recognized if it is not taken, in time and in space. These conditions vary with the year, with the season, with weather conditions, and with the time of day; they vary in different habitats, in different strata, with different kinds of soil and against different backgrounds, and in different kinds of vegetation. There are many difficulties in the way of estimating even relative numbers of different species. Less abundant species are likely to be confused with more abundant kinds. There are differences in appearance, in conspicuousness, in degree of activity, in ordinary behavior and behavior upon being disturbed, with different species, which make some of these much more readily perceived, or recognized, or captured, than others. Differences in time of appearance and activity, and in place, in surroundings, may make certain species appear more or less abundant or frequent than they really are.

The method used by Shull ('11: 218) in estimating numbers of grasshoppers per unit of area is valuable, but does not distinguish the different species. His suggestion "to collect immense numbers, and depend upon majorities to decide the usual habitat," besides being impracticable, is not satisfactory, for the numbers collected are not a true index of the numbers which actually occur by reason of the difficulties mentioned above.

The Method Used in Determining Frequency and Abundance.—The method used by the writer was to visit the various habitats, taking one or several grasshopper specimens of each species for verification, and to estimate abundance not from the numbers collected, but from the numbers observed per unit of area in each station, having in mind the considerations which tend to over- or underestimation of actual numbers.[1] If in the bracken-blueberry growth, a considerable number of *Melanoplus angustipennis* could be seen nearby, within a rod or two, and if this condition was practically uniform over the area of the association, as nearly as could be seen, an infinity sign was put down in the field notes opposite its name, indicating numbers in which it occurred, followed by the number collected, thus (∞, 2). If in twenty minutes walk through the aspens only four or five specimens of *Scirtetica marmorata* were seen, and two collected, the notes would appear thus (*s*, 2), the letter *s* representing *several.* If in a half-mile of sandy roadway, a considerable number of specimens of *Spharagemon* were seen, but never close enough together to be abundant, the numbers would be indicated by the plus sign (+, 1). If a species was taken in a particular kind of habitat in nearly all stations of this habitat visited, or if it was regularly to be found in extensive areas representing this habitat, its frequency for that habitat was considered to be high. From the records which thus accumulated it was possible to arrive at a relative estimate of frequency and abundance for each species in its various habitats. Though the method is not free from error, it is the best which was available, and it is believed to be not far from a representation of actual conditions. Terms expressing degrees of frequency and abundance are *dominant*, *abundant*, *frequent*, *occasional*, *infrequent*, and *accidental*. These terms are used in the table of distribution on p. 153, and are there defined.

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[1] Facilities for determination were available at the biological station, and the species were identified as they were taken. With most of the species the writer had been acquainted, and the species soon became so familiar as to be recognizable at sight. In life there are many rather conspicuous peculiarities of color and of behavior which aid in recognition. Whenever there was doubt as to identity, as in the case of *Melanopli* particularly, specimens were caught and examined critically in the field, to avoid overlooking species of similar appearance. In all cases one or several specimens of each species were kept for verification. There is no reason why many familiar insects should not be recognized at sight as easily as birds are identified by ornithologists.

The Relation of the Animal to Plant and Animal Communities.

The Plant Association as an Index of the Habitat.—The area of the habitat as considered in this study has already been defined as the area conveniently marked by the extent of the plant association. In the plant association or plant community physical conditions and vegetation are generally uniform. In the early stages of development of vegetation, local physical conditions dominate. In later stages the vegetation assumes the type determined by climatic conditions, and exerts nearly complete control over local physical factors. Thus the grasshoppers in early stages of vegetation, as the *Œdipodinæ*, most of which live in very open grassland, are more intimately associated with physical conditions, which to them are more important, while those of advanced stages of vegetation depend more upon vegetational factors, and less upon the character of the soil. *Melanoplus islandicus*, associated with climax deciduous forest, is a species of the second type. The grasshoppers as a group are most abundant in early stages of vegetation in forest climates, while in grassland climates they occur abundantly in all stages. The plant association may thus be taken as the index of environmental conditions; it expresses the resultant of physical and vegetational conditions to which animals are subjected. Within the area of the plant association local variabilities in physical conditions are usually accompanied by local variabilities in the vegetation; the latter may exist independently. Grasshopper species are affected by these local differences.

Vertical Distribution.—The distribution of terrestrial animals in space is both vertical and horizontal (Shelford, '12*b*). Most of the grasshoppers of the Douglas Lake region belong to the ground and field (herbaceous) strata, as do the *Acridiidæ* in general. With the exception of the forest species which oviposit in wood, the eggs are laid in the soil. Most of the species require bare soil, and many of their activities take place directly upon the surface. *Stenobothrus* is more commonly seen upon the plants, and certain species of *Melanoplus* live upon the plants part of the time. *Podisma glacialis variegata* is a shrub-inhabiting species. Grasshoppers are in general typical animals of the ground stratum. Other families of *Orthoptera* are more typical of other strata.

Horizontal Distribution.—Within a region an animal species will select habitats or associations in which conditions of the optimum environment are most closely approximated. The table on p. 153 indicates that no two grasshopper species of the Douglas Lake region select the same set of environmental conditions. Certain of the species are similar in distribution, but none are identical. They replace one another in different habitats, with some overlap, and can be arranged in series according to gradients of environmental factors (Fig. 1, p. 156).

Although a single species may be found in more than one association, it is not equally abundant nor equally regular of occurrence in these associations. Certain of the grasshopper species are typical of only one association, as *Melanoplus fasciatus* in closed aspen forest. *Melanoplus atlanis*, the most generally distributed species, though abundant in five habitats, is most abundant in ruderal grassland, and very typical in such situations. Other grasshoppers are *more* characteristic of the other four associations in which *Melanoplus atlanis* is abundant.

The various habitats in which a particular species may be found happen to be more or less similar in physical and vegetational conditions. Data in the table on p. 153 indicate that those associations in which a grasshopper is found in common may agree only in containing certain or all of the conditions necessary for that species, and that there need be no successional or geographic relationships between the associations, nor is it a matter of concern whether the associations be native or ruderal, extensive or local.

The occurrence of a grasshopper species, or of several species, in two habitats or associations does not mean that these furnish similar environmental complexes for animals. It means simply that certain environmental conditions necessary for these particular species are *included* among the conditions provided by these habitats. Thus in the Douglas Lake region, *Melanoplus islandicus* is found in beech-maple forest and in the cedar-bog forest. These two associations are radically different in many respects, as habitats for animals. The entire range of conditions presented within the area of an association, particularly if it be extensive, is likely to be considerably more inclusive than the range of conditions required by many animal species. Taxonomic groups of animals which are affected more particularly by conditions *differing* in the two habitats, will be represented differently in them. It follows that very little reliance can be placed on comparisons of habitats on the basis of the study of a taxonomic group, such as grasshoppers, except in respect of the particular conditions critical to the species of this group. Comparisons of the entire animal communities of the two habitats would not be subject to this limitation, since nearly all of the environmental conditions within the two habitats would come into consideration.

Within any one association the animal species may be distributed generally throughout its area, as *Melanoplus angustipennis* in the bracken-blueberry growth; in certain instances it may be restricted to a part of the area characterized by a slight environmental difference; or it may occur in scattered parts of the association, characterized by scattered local differences, as *Scirtetica marmorata* in the lichen-covered patches within the bracken-blueberry growth.

The Animal Environment.—In addition to the physical and vegetational influences upon the animal species, that of its animal environment must also be recognized. Direct effects of the animal-environment upon the animal species are probably greater than the indirect effects produced by modification of physical and vegetational environments. Among these latter more general effects of the animal community are the accumulation of organic remains, and particularly the effects of phytophagous animals upon vegetation. It is probable that animal environmental influences are greater than is commonly supposed, but that ordinarily they play a subordinate part as compared with physical and vegetational influences (cf. Shelford, '12*a*: 94).

The grasshopper species is not only affected by the animal environment, but is itself a part of it. So far as the relations of the grasshoppers to the association and with other species is concerned, about all that is known, in general, is that they are among the most important of the plant-eating animals of grassland associations, in which some of them are dominant species; and that they form the principal food supply for a comparatively large number of predaceous and parasitic enemies. It is not known to what extent different grasshopper species compete with one another. Differences in habitat and in time of activity may indicate removal of competition among certain species.

Successional Relations.

The successional relations of the Douglas Lake associations have been discussed by Gates ('13: 48), and a diagram illustrating the successions is included. The work on the grasshoppers has not covered so wide an area, consequently many of Gates' associations are not well represented. Fig. 1, on p. 156, will serve to illustrate successional relations of the associations in which grasshoppers were taken, and the changes in grasshopper species may also be seen, as one plant association is replaced by another. Initial stages in dry soil are shown at the lower left-hand part of the diagram; initial stages in wet habitats at the lower right. The two series converge at the top of the curve, in the climax beech-maple forest. The ordinary course of succession from marsh associations is toward bog forest; from beach grass into pine forest. These successions are not shown in the diagram. The aspen association is the result of secondary succession from pine forest, which is no longer well represented in the immediate region. Grassland associations between beach-grass and wet meadows are represented only by ruderal growths; which have originated mostly by secondary succession. Closed grassland is not represented, for invasion by forest occurs before it can develop.

Only the series leading from bare sand to climax forest through the aspen stages of burnt-over pine lands will be discussed in relation to grasshopper succession. The following table, taken from the distribution table on p. 153, and with the same notation, shows the grasshopper species of the various stages. Accidental and atypical occurrences are not recorded. It should be remembered that the bare soil habitat is never extensive in the region, and that grasshoppers of bare soil depend also upon nearby vegetation.

TABLE II.

	Bare Sand.	Beach-Grass.	Bracken-Blueberry.	Closed Aspens.	Beech-Maple.
Dissosteira carolina	Freq.[1]	Freq.	Occas.		
Spharagemon bolli	Freq.[1]	Occas.	Occas.		
Circotettix verruculatus	Freq.	Occas.	Occas.		
Arphia pseudonietana	Freq.[1]	Infreq.	Infreq.		
Hippiscus tuberculatus	Freq.	Infreq.	Infreq.		
Camnula pellucida	Freq.[1]	Freq.[1]	Freq.[1]		
Melanoplus angustipennis	Occas.	Freq.	Dom.		
Melanoplus luridus			Occas.		
Scirtetica marmorata			Infreq.[2]		
Melanoplus fasciatus			Infreq.	Freq.[1]	
Chloealtis conspersa			?	Infreq.	
Podisma g. variegata					?
Melanoplus islandicus					Freq.
Melanoplus atlanis	Freq.[1]	Abund.	Freq.	?	Infreq.

From examination of the table we see that there is a successive change of species with development of vegetation, and that even when species are not replaced with changes in associations, their numbers are successively increased or decreased.

The succession from bare sand to closed hardwood forest includes two successional series, the development from very open grassland growth to closed grassland, and the development of closed forest from open forest growth. The transition stage is the open aspen forest, in which trees are small and scattered. Ground conditions, dominated by the bracken-blueberry cover, are those of grassland which has not yet reached the closed stage. The change in ground conditions from those of grassland to those of closed forest is radical, as shown by the great difference between the grasshoppers of the bracken-blueberry and closed aspen stages. Climax beech-maple conditions have probably not yet been fully developed, in the immediate region, from the closed aspens of the pine lands. The beech-maple forest of the morainic areas of the region represent the ultimate condition of present closed aspen areas.

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[1] Frequently found, usually not in numbers, but in a few places approaching abundance.

[2] More typical of scattered patches of lichen-covered surface; distribution in bracken-blueberry not continuous.

The grasshoppers of the table show certain likenesses and differences in habitat-selection which may be correlated with their behavior characters. The first five species, all typical of very open situations, are active, motile forms, of strong and sustained flight, and are usually seen resting upon bare soil. They lay eggs usually in soil of loose texture. They are frequently seen on roads, and patches of bare soil, in the Douglas Lake region, and are abundant near the beach. They are frequently seen in the interstices between plants in open grassland, and become successively less numerous with the closing of the vegetation.

Camnula pellucida is less like the typical bare-ground *Œdipodinæ* in behavior. It is more variable in distribution, and though practically restricted to grassland, is more numerous in ruderal growths.

Melanoplus angustipennis is an interstitial species, and increases in abundance with the closing of the vegetation until the sand is relatively stable, with the admixture of a little humus, though the soil is still loose in texture.

Melanoplus luridus and *Scirtetica marmorata* have not been found in sufficient numbers to determine their status with satisfaction. The former appears to be a species of nearly closed grassland; the latter is more or less closely associated, in this region at least, with lichen surfaces that had not developed in earlier stages of herbaceous growth.

Melanoplus fasciatus and *Chloealtis conspersa*, in this and other regions, are associated with dry open forests and forest borders. They are more frequently short-winged, and exhibit a departure from grassland behavior. The latter is known to deposit eggs in wood.

Melanoplus islandicus is a shade-dwelling species of deep woods. It is flightless, and probably lays eggs in wood. *Podisma* *glacialis variegata* has not been taken in the beech-maple forest, but probably occurs there. It differs from the other grasshoppers of the region in being of the shrub stratum, rather than of the ground or herbaceous strata.

The species in the table are arranged approximately in the order of succession of the associations in which they occur. *Melanoplus atlanis*, being more generally distributed, can hardly be assigned to a particular place in the series, and is placed at the bottom accordingly. It is most abundant in ruderal grassland, and is not at all common in forest.

A parallel development of vegetation in sand is seen in a region in central Illinois. The herbaceous vegetation is sand prairie, is very open in the initial stages, and is replaced by xerophytic oak forest before a closed grassland is reached. The habitat-relations of the various grasshopper species are discussed by Hart ('07). A number of the species of the Douglas Lake region are here represented in habitats occupying equivalent stages in the successional series.

The initial stages of herbaceous growth, characterized by a large proportion of bare surface, are accompanied by active, very motile grasshoppers which rest normally on the surface and which lay eggs in soil of loose texture. With the closing of the herbaceous growth grasshoppers of this habit gradually decrease in numbers, giving way to species which rest part of the time upon the vegetation, which are less motile, and which lay eggs in the less sterile types of soil of such situations. In sand regions of the forest climate, usually, forest invasion occurs before the herbaceous growth becomes closed. The ground conditions remain those of grassland until the forest approaches the closed stage, when the grasshoppers of open grassland are abruptly replaced by forest grasshoppers, which are less motile, usually flightless. They are fewer in species and individuals than grassland members of the group, in part because of the fact that in advanced forest stages, in which the ground is almost entirely covered with dead leaves, the soil is generally inaccessible for egg-laying, and oviposition takes place typically in wood.

Geographic Relations.

The vegetation of the region, as has already been mentioned, is composed of two geographic elements, that of the Northeastern Conifer Province and that of the Eastern Deciduous Province. In addition there are certain associations, chiefly made up of plants which cannot be assigned to these vegetation regions. Comparison was made of the geographic distribution of grasshoppers with that of the vegetation, and with their own local distribution.

Most of the species are generally northern in distribution. Of these *Podisma glacialis variegata*, *Melanoplus islandicus*, and *Chloealtis conspersa* may be assigned to the Northeastern Province. *Circotettix verruculatus*, *Melanoplus fasciatus*, *M. luridus*, *M. minor*, *Camnula pellucida*, and *Stenobothrus curtipennis* range in both northeastern and northwestern coniferous regions, extending south to varying distances in both Appalachian and Rocky Mountains. *Arphia pseudonietana* does not range so far to the east as these preceding species. *Arphia*, *Melanoplus luridus*, *M. minor*, *Camnula*, and *Stenobothrus* occur also throughout the northern part of the Prairie Province.

Spharagemon bolli and *Scirtetica marmorata* may be assigned to the Eastern Deciduous Province. *Hippiscus tuberculatus* is found in the northern parts of both prairie and deciduous forest. *Melanoplus angustipennis* is a prairie species, being found abundantly in sandy parts of the Prairie Province, ranging also west into the sage-brush country, in Utah.

Dissosteira carolina, *Melanoplus atlanis*, *M. femur-rubrum*, *M. differentialis*, and *M. bivittatus* are of very wide geographic distribution, ranging over most of the United States and much of Canada.

Certain species of the first group are rather sharply restricted to the coniferous forest regions, while others range well into the prairie and deciduous forest regions. *Chloealtis* and *M. fasciatus* range also into deciduous forest. *M. luridus*, *M. minor*, *Camnula* (in the northern prairie states), and *Arphia* range also into the prairie region. An excellent instance of the sometimes sharp boundary between two provinces is shown along the foothills of the eastern Rocky Mountains in Colorado. Here *Chloealtis*, *Circotettix*, *M. fasciatus*, and *Camnula* are not found outside of the mountains, while *Arphia*, *M. luridus*, and *M. minor* are found on the plains. *Stenobothrus* bears no particular relation to climatic vegetation regions, as its presence is determined by local conditions of moisture.

The occurrence of *Melanoplus angustipennis* at Douglas Lake, so far from the prairie region, seems at first very unusual. So far as the writer is aware, the species is not recorded from Michigan, though it must occur all along the Lake Michigan shore, and is known from Ontario. Its most necessary environmental condition is sandy soil, and this is well developed along most of the shore-line of the Great Lakes.

The five species of most wide distribution geographically are those which have least definite habitat-preferences within a particular area. The extensive range, and the fact that these particular species are among those most important economically, as destructive to crops, is to be explained in terms of tolerance of widely varying conditions.

The species which are restricted to forest habitats in the Douglas Lake district are restricted geographically to forest provinces. Certain of the species found in grassland or in open forest in the region studied range outside the forest provinces, being thus clearly independent of forest growth, while others appear to be restricted to forested regions, though found in open habitats. It is probable that *Camnula pellucida* and *Circotettix verruculatus* are restricted to the mountain areas of Colorado by radical changes in physical conditions from mountains to plains, rather than by changes in vegetation.

In general, it may be said that grasshopper species associated with the climatic plant association have the same geographic range as this association. This range, when shared by many species, determines the ecological province. Where two similar climatic vegetation regions adjoin, as in the case of northeastern and northwestern conifer regions, the same grasshopper species may range over both, in similar associations. Certain species may also range locally into other provinces, in habitats locally approximating those of their own climatic association. Species associated with local habitats may be restricted to the province, but more usually range over much wider areas (Shelford, '11: 606). Species of very general local distribution within a restricted area are likely to be very generally and very widely distributed geographically, and are the species which most frequently invade ruderal and cultural growths, and which tend to replace other species with the spread of civilization. Species of closed associations show more evident local and geographic relation to the vegetation than species of open associations, in which *local* physical conditions dominate (cf. Shelford, '12*a*: 89, 90).

Seasonal Relations.

Distribution of animals in space is more or less affected by distribution of animals in time. In dealing with the latter it seems that seasonal relations and life-histories of animals are comparable to habitat relations and habitat-preferences, when dealing with distribution in space.

The growing season is not uniform in physical and vegetational features of the environment. Seasonal changes are conveniently marked by changes in *aspect* of the vegetation. The successive changes in the environment for grasshoppers in the course of a season are somewhat different in forest associations from those in grassland.

A field of study of seven weeks is not long enough to determine seasonal relations of grasshoppers, nor to determine what species of grasshoppers occur in that region. *Chortophaga viridifasciata* (De Geer), an early spring grasshopper almost certainly occurs at Douglas Lake. *Melanoplus scudderi* (Uhler) and *Melanoplus punctulatus* (Uhler) may reasonably be expected to occur within the region. Both are short-winged forest-inhabiting species of late summer and autumn.

The time of adult activity of such species as were found, however, is known; the species may be arranged according to life-history into the following groups:

1. Species which hibernate as nymphs, appearing as adults in spring, remaining active only during early summer, represented by *Hippiscus tuberculatus*.
2. Species which hibernate as eggs, maturing very early in summer or late in spring, becoming scarce or disappearing by about September 1. *Melanoplus minor*, *M. bivittatus* (sometimes persisting in numbers till frost), *M. fasciatus* (this species may more properly belong in the fourth group).

3. Species which mature in spring or early summer, but which remain abundant until frost. *Chlœaltis conspersa, Melanoplus angustipennis, M. atlanis, M. femur-rubrum.* The last two are known to be two-brooded, and *M. angustipennis* probably is also, in parts of its range.
4. Species which mature in early July, remaining active until frosts. This is the common grasshopper life-history. *Stenobothrus curtipennis, Camnula pellucida, Dissosteira carolina, Spharagemon bolli, Scirtetica marmorata, Circotettix verruculatus, Melanoplus islandicus, M. luridus.*
5. Late-maturing species, appearing from late in July to the middle of August, and remaining until frosts. *Arphia pseudonietana, Podisma glacialis variegata, M. differentialis.*

The different timing of the period of activity of the species may be correlated with one or both of two kinds of seasonal difference in environmental influence: (1) Changes in physical and vegetational conditions necessary for the various activities. Conditions of temperature, moisture, vegetation, etc., change so materially with the season as to present very different environments for animals at different times of the year. Grasshoppers of different habitat-preferences may perhaps occupy the same area at different seasons. Life-histories are affected by geographic variation of climatic conditions. The entire complex of behavior characters of the animal is intimately associated with its life-history (Shelford, '12*b*: 334). (2) Seasonal differences in antagonistic influence of other animals, particularly those of similar requirements. These influences among animals which eat the roots of the corn plant and the strawberry plant are discussed by Forbes ('09: 296). Different species of certain insect genera, among them *Arphia* and *Hippiscus*, which are of similar habits and which occur in the same association, sometimes are active during successive parts of the season. One species of a genus may be completely replaced by another in a very short time, the second species continuing abundantly during the remainder of the season (Vestal, '13*b*). This replacement of one insect by another of similar habits is not uncommon among species of one genus, though not confined within genera. The differences in time of appearance among all the grasshopper species within a region, with the result that within some of the associations fewer species occur together at one time, may perhaps be accounted for partly in terms of antagonistic animal influence. The fact that grasshopper species of unique habits, which occupy certain habitats by themselves, so far as grasshoppers are concerned, have the most common type of life-history, hibernating in the egg stage and active from early July till frosts, suggests that antagonistic influence is not a factor in determining *their* life-histories. Such species are *Stenobothrus curtipennis* in marshy growths, and *Melanoplus islandicus* in deep forests.

It appears that the species with least definite habitat-preferences (*Melanoplus atlanis*, *M. femur-rubrum*, *M. bivittatus*) have also least definite life-histories. They mature early, remain active till frosts, and are sometimes two-brooded. Their wide distribution in time is probably due to the same cause as their wide distribution in space, namely, their lack of responsiveness to slight differences in environmental conditions.

V. Summary.

Distribution of grasshopper species within the region studied bears evident relation to the plant associations. The plant association is the index to environmental conditions, and its extent marks the area of the habitat. In studies of local distribution within a region, all the plant associations, including local and ruderal associations, should be considered, since otherwise certain habitat relations may be overlooked.

As an environmental complex, the plant association is usually more inclusive than the total of conditions required by a particular species, particularly if it is climatic or extensive. Associations may be similar as environments for grasshopper species if they agree merely in including physical and vegetational conditions critical to those species. Grasshoppers select habitats or associations in which favorable conditions are to be found, irrespective of past history, extensiveness, geographic or successional relationships of the vegetation. There is very seldom any direct relation between grasshopper species and species composition of the plant associations, as few grasshoppers are selective feeders.

Most of the grasshopper species are of the ground stratum, and soil conditions are essential. These grasshoppers are typical of open herbaceous associations, characteristic in initial stages of vegetational development in dry ground. Species of open forest are mostly grassland forms, since ground conditions are essentially the same in both situations. In closed forest and moist herbaceous associations species and individuals are less numerous, are not confined to the ground stratum, and are more intimately associated with vegetational conditions. Grasshopper species can be arranged according to gradients of environmental factors (Fig. 1, p. 156).

Grasshopper succession is incidental to the development of vegetation. The change is not only one of species, but of habits as well.

Grasshopper species have in general the geographic range of the types of associations which include the necessary physical and vegetational conditions. The ranges of many species are in agreement with the areas of vegetation provinces. Species of least definite local distribution are widespread geographically.

Seasonal differences in time of activity of grasshopper species are marked. This is probably partly due to antagonistic influence of other animals. Seasonal and local distribution are interrelated. Species of indefinite local distribution have also least definite time distribution.

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THE ASEXUAL CYCLE OF PLANARIA VELATA IN RELATION TO SENESCENCE AND REJUVENESCENCE.

C. M. CHILD.

I. The Life Cycle under Natural Conditions.

During early spring in the region about Chicago, a planarian appears in temporary ditches and pools, particularly in those which are more or less filled with dead leaves. It is also often found in permanent bodies of water such as springs, permanent ponds and brooks, but seems to attain the greatest numbers in the temporary ditches and pools. The animal is apparently the species recently described by Stringer ('09) and named *Planaria velata*. The shape and proportions of the larger individuals are indicated in Fig. 1.

When the animals first appear soon after the ice melts they are mostly only 2–3 mm. in length and commonly light in color. They grow rapidly and soon the dorsal surface becomes very deeply pigmented so that they appear almost black. They are very active and their locomotion is much more rapid than that of most other fresh water planarians. During this period they react readily to meat of various kinds and can be collected in large numbers by placing pieces of meat in the water. In about four weeks they attain a length of 12–15 mm., their movements gradually become slower, they cease to react to food, become light gray in color from loss of pigment and sooner or later the pharynx disintegrates.

Within a few days after these changes a process of division begins. As the worms creep about, the extreme posterior end adheres to the substratum and the rest of the animal pulls away and leaves it behind as a small fragment which becomes more or less spherical and within a few moments is covered with a slime which adheres to the underlying surface and hardens into a cyst. This process of division is repeated, often several times within a few moments, so that as the animal moves across the containing vessel it may leave behind it a series of such pieces. The pieces vary considerably in size, some being as large as 1.5 mm. in diameter, some only about 0.5 mm. The process continues until half or two thirds or sometimes even more of the worm is separated into pieces and then the anterior region including the head may encyst without further division or in some cases dies.

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Under natural conditions the encysted pieces remain quiescent during the summer and the following winter and in early spring emerge from the cysts as minute, very active worms which at once begin to feed and grow and repeat the cycle. As I have determined by experiment, the encysted pieces are not capable of withstanding desiccation and it is probable that this fact is connected with the occurrence of the worms in ditches and pools partly filled with dead leaves. In such localities even though the water disappears, the bottom under the thick layer of leaves is always more or less wet and the encysted pieces are not subjected to drying.

During the last thirteen years I have collected these worms almost every year and have never found a single individual with mature sexual organs or even any indication of sexual reproduction. Every year the active period ends with decrease in activity, cessation of feeding, loss of pigment, fragmentation and encystment of the fragments.

In this species then, under the conditions where it occurs in this locality, development and growth result in a process of senescence, the individual breaks up into fragments which undergo regulation within the cysts to small whole animals, and these are to all appearances physiologically as well as morphologically young and are capable of repeating the life cycle. In short, senescence is followed in these animals not by death but by asexual reproduction and rejuvenescence.

During a number of years I have kept a stock of these worms in the laboratory, have bred them through several asexual generations and have subjected them to various experimental conditions. The results of this asexual breeding and the experimental modifications of the life cycle will be discussed in another paper.

II. The Physiological Resistance to Depressing Agents of Young and Old Worms.

In these experiments the method of comparing resistances which I have called the direct method was used. Here the depressing agent is used in sufficiently high concentration to kill the animals within a few hours and the occurrence of death is determined by disintegration of the worms which begins within a few moments after death. This method has been fully described in another paper (Child, '13*a*). In that paper it was shown that with this method the animals with the higher rate of metabolism or more strictly of cell respiration are less resistant and therefore die and disintegrate earlier than those with the lower rate. Thus the differences in resistance enable us to compare the rates of respiration and so in a general way the rates of metabolism.

In Table I. the first vertical column gives the length of time in the depressing agent in hours and minutes, the second the serial numbers of the lots of worms compared, and the columns I.–V. under "Stages of Disintegration" give the number of worms of each lot in each stage of disintegration at each time. As regards the five stages, I have found it convenient to distinguish more or less arbitrarily these stages in the process of disintegration, for disintegration usually appears first in certain definite regions of the body while other regions are still alive and show movement and it follows a more or less regular course (Child, '13*a*). The five stages are briefly characterized as follows:

I. Intact, no disintegration.

II. Disintegration beginning, usually in head region.

III. Body beginning to disintegrate but form still retained.

IV. Margins disintegrated, form disappearing in consequence of swelling of tissues and separation of cells.

V. All epithelium and pigment gone; swelling of tissues has extended to all parts and original form has disappeared.

The distinction of these stages makes it possible to compare different lots of worms more closely than if only the time of complete disintegration were noted.

In Table I. Lot 1 consists of ten worms 1.5–2 mm. in length, which had emerged from cysts within three or four days preceding and had been fed once with pieces of earthworm after emergence. Lot 2 consists of ten worms 13–15 mm. which had been raised in the laboratory from cysts with earthworm as food and were almost ready to encyst again.

Table I.

Series 77. KCN 0.001 mol.

Length of Time in KCN.	Lots.	Stages of Disintegration.				
		I.	II.	III.	IV.	V.
1.30	1	8	1	1		
	2	10				
2.00	1	5	1		1	3
	2	10				
2.30	1	4	1			5
	2	10				
3.00	1	2	2	1		5
	2	10				
3.30	1			3		7
	2	6	3	1		
4.00	1					10
	2	3	5	1	1	
4.30	2		8	1		1
5.00	2		4	4	1	1
5.30	2		1	4	3	2
6.30	2				3	7
7.30						10

It is evident at once from the table that the resistance of the worms of Lot 1 recently emerged from cysts is very much less than that of the large worms of Lot 2. Disintegration begins in

Lot 1 after one and one half hours in KCN, while the worms of Lot 2 are still intact and slowly moving about. In Lot 2 disintegration begins after three hours. All the worms of Lot 1 are completely disintegrated after three and one half hours, those of Lot 2 after seven and one half hours, *i. e.*, the survival time of Lot 2 is nearly double that of Lot 1. In other words the worms of Lot 1 have a much higher rate of metabolism than those of Lot 2.

That the difference in size of the worms is not responsible for the difference in survival time is evident for two reasons: first in these flattened elongated animals the surface increases almost as rapidly as the volume and second the time of beginning of disintegration (Stage II.) is much later in Lot 2 than in Lot 1. The earliest stages of disintegration involve the external surface of the body and the surface of the large worms including the cilia remains alive for a much longer time than that of the small worms. Moreover, if the difference in size determined the difference in survival time we should expect that this would be much greater since the small worms are only a minute fraction of the size of the larger. The difference in the rate of the metabolic processes affected by the KCN is the only factor which will account for the results (Child, '13*a*).

Unfortunately it has thus far been impossible to compare the worms emerging from the cysts with young worms hatched from eggs because I have never observed sexual reproduction in this species, but the difference in rate of metabolism between the small and large worms is similar to the difference known to exist in other forms between young animals sexually produced and old.

It is, however, not necessary to use the extremes of the life cycle for comparison. Animals in various stages of growth may be compared and in all cases those which are nearer the stage when encystment occurs, *i. e.*, those which are older as regards growth and development, show the higher resistance.

In Table II. the survival times of a series consisting of five worms 5-6 mm. in length (Lot 1) and five worms 11-12 mm. (Lot 2) are given.

Table II.

Series 64, I., II. KCN 0.001 mol.

Length of Time in KCN.	Lots.	Stages of Disintegration.				
		I.	II.	III.	IV.	V.
2.30	1	2	3			
	2	5				
3.00	1		3	1	1	
	2	5				
3.30	1		1	2	1	1
	2	3	2			
4.00	1				1	4
	2	1	4			
4.30	1					5
	2		4	1		
5.30	2			5		
6.30	2				4	1
7.30	2					5

In this series also the younger worms show less resistance, which signifies a higher rate of metabolism, but a comparison of Table II. with Table I. shows that Lot 1 of Table II. has a longer survival time than Lot 1 of Table I., *i. e.*, worms of 5–6 mm. in length have a lower rate of metabolism than worms recently emerged from cysts. These facts show that a progressive decrease in the rate of metabolism occurs during the growth of the animals. Those newly emerged from cysts have the highest rate, those which are full-grown and nearly ready to fragment and encyst have the lowest rate, while intermediate stages show rates between these two extremes.

These results have been confirmed by various other series with both KCN and alcohol. The small newly emerged worms die much earlier in all cases than the large worms. The differences in resistance to KCN, alcohol, etc., between young and old animals are the same in animals freshly collected from their natural habitat as in animals bred for one or more generations in the laboratory.

The results obtained in this way are further confirmed by the much greater activity of the small recently emerged animals. They move much more rapidly, are much more irritable and show a much higher rate of growth than the large animals. And finally the small worms from the cysts are capable, as noted in the preceding section, of repeating the life cycle. There can I think be no doubt that the worms emerging from the cysts are physiologically young and that they undergo a process of senescence as they grow in size. Evidently a process of rejuvenescence is associated in some way with the asexual reproduction which follows growth and development.

III. Experimental Reproduction.

1. *The Course of Experimental Reproduction.*

The process of reproduction of whole animals from pieces isolated by section is very similar to that in other planarians. Pieces from any region of the body and above a certain limit of size, which varies somewhat with the region, are capable of giving rise to whole animals.

As in other species of Planaria, the process consists in part of the outgrowth and differentiation of embryonic tissue from the cut surface and in part of redifferentiation of other tissues to a greater or less distance from the cut surface. In pieces of equal length the amount of anterior new tissue is greater and of posterior new tissue less in those from the anterior region of the body, while with increasing distance of the end of the piece from the head region the amount of anterior new tissue increases and

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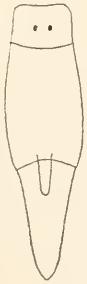
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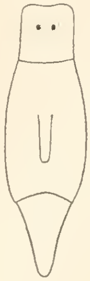
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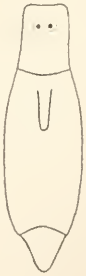
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that of posterior new tissue decreases. The development of the new head is more rapid in anterior than in posterior pieces. The position of the new pharynx is posterior to the middle in anterior and anterior to the middle in posterior pieces. Short pieces from the extreme anterior region frequently fail to develop a new posterior end. Fig. 2 shows a piece of this kind. Figs. 3, 4 and 5 show three pieces, the first from the anterior, the second from the middle and the third from the posterior region. The different amounts of new tissue produced are seen in the figures. All these graded differences, like those in *Planaria dorotocephala* (Child, '11*c*), indicate the existence of a physiological gradient of some sort along the axis. As a matter of fact this gradient is essentially similar to that which exists in *P. dorotocephala* (Child, '12, '13*c*).

2. *The Encystment of Artificially Isolated Pieces in Relation to Size of Piece and Region of Body.*

Pieces isolated by section may undergo the regulation to whole animals either with or without encystment. The frequency of encystment varies with region of the body from which the piece is taken, with the size of the piece and with the physiological age of the animal. The following records of series will serve to illustrate this. In these series a number of worms, ten, twenty or twenty-five, from the same stock and as nearly as possible of the same size and in the same physiological condition are cut into a number of as nearly as possible equal pieces, the corresponding pieces are placed together in one lot and results recorded for each piece. Since different numbers of worms are used in different series the results are given in percentages.

Series 19, April 13, 1911.—Ten worms, full grown (12–14 mm.), but still feeding and deeply pigmented. Heads removed and remainder of body cut into two equal pieces, *a*, the anterior, and *b*, the posterior. Table III. shows the percentages of the pieces which develop into whole worms without encystment and of pieces which encyst soon after the operation and emerge, from a few days to several weeks later, as whole worms after regulation in the cysts.

TABLE III.

	No Encystment.	Encystment.
a	90	10
b	60	40

Series 20, April 13, 1911.—Ten worms from same stock, of same size and in same condition as Series 19. Heads removed and body cut into four equal pieces, *a*, *b*, *c*, *d*, *a* being the most anterior. Table IV. gives the results.

TABLE IV.

	No Encystment.	Encystment.
a	90	10
b	80	20
c	30	70
d	20	80

Series 27, April 17, 1911.—Ten worms like those of Series 19 and 20 in size and condition. Heads removed and body cut into eight equal pieces, *a–h*, *a* being the most anterior. The results are given in Table V.

TABLE V.

	No Encystment.	Encystment.
a	20	70
b	10	90
c		100
d		100
e		100
f		100
g		100
h		100

It is evident from these three series and abundantly confirmed by numerous others, first, that the frequency of encystment of pieces increases from the anterior to the posterior end of the body and second, that the frequency of encystment increases as the size of the piece decreases. In all these series the greater frequency of encystment in more posterior pieces is evident in greater or less degree. In Series 19 where the pieces represent halves of the body the percentages of encystments are small, in Series 20, composed of $\frac{1}{4}$ pieces, they are larger except in the most anterior piece and much larger in the two posterior pieces *c* and *d* which together equal *b* of Series 19. And finally, in Series 27 which consists of $\frac{1}{8}$ pieces all the pieces encyst except 30 per cent. of *a* and 10 per cent. of *b*. When the pieces are cut still smaller all encyst.

The frequency of encystment then shows in pieces of equal size a gradation from the anterior to the posterior end of the body and indicates the existence of some sort of a physiological gradient in the animal. Encystment may, however, occur in pieces from any region if they are sufficiently small, but in general anterior pieces must be smaller than posterior pieces to give the same frequency of encystment. This fact indicates that the physiological state of the piece differs in some way with its size. As a matter of fact this species possesses essentially the same sort of gradient in rate of metabolism as *Planaria dorotocephala* (Child, '13*a*, '13*c*) and the relation between frequency of encystment, region of the body and size of the piece depends upon the existence of this gradient and the changes in rate of metabolism which occur in pieces from different regions of the body and of different size after isolation. Further consideration of these points is postponed to another time.

3. *The Frequency of Encystment of Pieces in Relation to Temperature.*

Series 53, October 5, 1911.—Animals 9–10 mm. in length were selected from a stock which had been kept at a temperature of 20° C., the heads removed and the bodies cut into four equal pieces *a–d*. Lots of ten each of each of the four pieces were placed in three different temperatures, 10°, 20° and 28–30° C. Table VI. gives the results in percentages.

It is evident at once from Table VI. that the frequency of encystment is greater with higher than with lower temperature, *i. e.*, the higher the rate of metabolism in the pieces the greater the frequency of encystment. Numerous other series give the same results without exception, not only for pieces, but for whole worms. Worms which have been kept at a temperature of 20°, when placed in a temperature of 30° will often encyst entire while at 20° they remain active until they fragment and the pieces encyst, and at 10° many of them do not encyst at all.

TABLE VI.

Temperature.	Pieces.	No Encystment.	Encystment.	Dead.
10°	*a*	100		
	b	90	10	
	c	20	80	
	d	10	90	
20°	*a*	100		
	b	70	30	
	c		100	
	d		100	
28–30°	*a*		100	
	b		100	
	c		100	
	d		100	

4. *The Frequency of Encystment in Pieces in Relation to Age.*

In the very small young worms recently emerged from cysts pieces, unless very small, usually reproduce whole worms without going through a period of encystment. As the worms increase in size and become physiologically older the frequency of encystment increases until in worms which are almost ready to fragment and encyst naturally all pieces resulting from section usually encyst.

The differences in this respect between half grown worms, worms which are about full grown but have not yet ceased to feed and still retain their dark color and worms which have stopped feeding and become gray in color are shown in the three series following.

Series 47, September 21, 1911.—Twenty worms about half grown (7 mm. in length) were cut into four equal pieces, *a–d*. The percentages of regulation without encystment and of encystments appear in Table VII.

TABLE VII.

	No Encystment.	Encystment.	Dead.
a................	95		5
b................	95	5	
c................	55	45	
d................	20	80	

Series 56 I, October 12, 1911.—Ten worms full grown but still dark in color and still feeding. Body cut into four equal pieces, *a–d*. Table VIII. gives percentages of encystments.

TABLE VIII.

	No Encystment.	Encystment.
a	60	40
b		100
c		100
d		100

Series 58 I, October 13, 1911.—Ten worms, full grown, gray in color and no longer feeding. Body cut in four equal pieces, *a–d*. Table IX. gives percentages.

TABLE IX.

	No Encystment.	Encystment.
a		100
b		100
c		100
d		100

The older worms show the greater frequency of encystment of pieces. The same results have been obtained in other similar series without exception.

5. *The Physiological Condition of Animals Reproduced from Artificially Isolated Pieces.*

The animals reproduced from pieces isolated by section are physiologically young, whether a period of encystment occurs or not. In this respect they are similar to the worms produced from the pieces which separate and encyst naturally. Small pieces cut from the bodies of old worms and allowed to reproduce whole animals show the same differences in rate of metabolism from old animals as the worms emerging from cysts naturally produced. The differences in susceptibility to cyanide are essentially the same as in Table I. Moreover, these small worms arising from pieces of large old worms are capable of rapid growth if fed and of repeating the life cycle. As they grow the rate of metabolism, as indicated by their susceptibility to cyanide, decreases, the rate of growth and the degree of activity also decrease, they finally stop feeding, lose their dark color and give rise to cysts again and from these a new generation of young worms emerges. Stocks of animals produced from pieces have passed through this cycle repeatedly in the laboratory.

The degree of rejuvenescence in this experimental reproduction varies with the size of the piece. The smaller the piece, the more extensive the reorganization and the younger the worm which results. In all respects these results are essentially the same as those obtained with *Planaria dorotocephala* and described in an earlier paper (Child, '11*b*).

It is evident also that there is no essential difference in this respect between the process of fragmentation in old worms and the reproduction of young worms from the encysted pieces in nature and the process of experimental reproduction of animals from pieces isolated by section. In nature the fragmentation occurs only in old animals by a process characteristic of a certain stage of the life cycle. In experiment the pieces can be isolated at any stage of the life history and may be of any size. In both cases the reorganization, together with the period of starvation which is also a factor as will appear, brings about rejuvenescence and the worms thus produced are capable of repeating the life history from the stage at which they begin again to feed to the stage of fragmentation.

IV. The Nature of the Process of Encystment.

It has been shown that the frequency of encystment of pieces increases with rising temperature, with decreasing size of the piece, with increasing distance of the level of the piece from the head region and with advancing age of the animals. Pieces from any region of the body may encyst if the temperature is sufficiently high, if the pieces are sufficiently small or if the animal is sufficiently old. All of these conditions must have something in common as regards their effect upon the pieces since all produce similar results. What is this common factor?

When a piece is cut from the body it is stimulated and its rate of metabolism increases. This is generally admitted but it can also be demonstrated by the cyanide method. The susceptibility to cyanide of a piece immediately after isolation is much greater than that of the corresponding region of the body in an uninjured animal of the same age and physiological condition. This greater susceptibility of the piece means that it has been stimulated by the act of isolation. After this sudden rise its susceptibility to cyanide decreases gradually during twenty-four hours or more and in small pieces may fall below that of corresponding regions in the uninjured animal (Child, '13*b*). This decreasing susceptibility means that the rate of metabolism in the piece is gradually decreasing as the stimulation resulting from section gradually disappears.

The cyanide method shows further that the degree of stimulation increases as the size of the piece isolated decreases and also as the distance of the level of the piece from the head region increases. In other words smaller or more posterior pieces are more stimulated by the act of section than larger or more anterior pieces. And finally pieces cut from worms at a higher temperature within certain limits are more stimulated and show a greater increase in rate than pieces from worms at a lower temperature.

These relations between the degree of stimulation of pieces and the factors of size of piece and region of the body and various external conditions have been worked out completely for *Planaria dorotocephala* and the data will be presented in full elsewhere. Sufficient work has been done on *P. velata* to show that the relations are essentially the same as in *P. dorotocephala*, but since the work on the latter species furnishes the foundations for the conclusions and since the data for that species are in more complete form and will be published in a short time the evidence for the above statements concerning the degree of stimulation in pieces of *P. velata* is not presented in detail.

So far then as region of the body, size of piece and temperature are concerned the frequency of encystment of pieces in *P. velata* runs parallel to the degree of stimulation by the act of section. Apparently the more the piece is stimulated by section the more likely it is to encyst.

The process of encystment in this species consists in the rapid secretion over the surface of the body of a thick slime which soon hardens into a tough membrane and forms the cyst. It is a familiar fact that stimulation is often followed in the turbellaria by the secretion of a large amount of slime. That is exactly what occurs in these pieces and in this species the slime hardens and forms the cyst. Apparently then the encystment of pieces in *Planaria velata* is simply the result of a sudden stimulation. Any factor that increases the stimulation increases the frequency of encystment.

As regards the greater frequency of encystment with advancing age of the worms, I have not been able to reach a definite conclusion based on experiment, but my observations indicate that old worms secrete more slime on stimulation than young. Apparently the gland cells either increase in number or the quantity of the substance in them which produces the slime increases as the animals grow older.

When the slime which produces the cyst first appears it is soft and an active whole animal is able to creep out of it without difficulty, but the pieces are much less active and do not succeed in escaping from it before it hardens. If the cysts are carefully opened with needles soon after they are formed and the pieces removed without injury or any great degree of stimulation they usually do not encyst again but develop into whole worms while free. But if they are injured or otherwise strongly stimulated they commonly encyst a second time.

In short all the facts indicate that encystment of pieces is merely a result of the stimulation accompanying section. It is not an adaptation to conditions or a preparation for the future in any sense. The animals do not encyst because they usually live in temporary bodies of water but they are able to live under these conditions because they encyst.

V. The Process of Fragmentation in Old Worms.

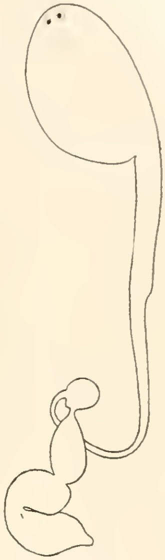
The process of fragmentation in nature is very evidently similar in character to the process of zooid-formation and fission in *Planaria dorotocephala* and *P. maculata* (Child, '11*e*). In consequence of increase in length of the body and the decrease in rate of metabolism as the animal becomes older the posterior regions of the body usually become to some extent physiologically isolated from the dominant region (Child, '11*a*, '11*d*).

That the occurrence of fragmentation is connected with a decrease in the rate of metabolism and consequent physiological isolatiion of posterior regions is clearly indicated by the fact that fragmentation may often be induced, even in worms which are not full-grown, by suddenly lowering the temperature ten to fifteen degrees. In such cases fragmentation usually begins in the posterior region within a few days.

The degree of isolation is not sufficient to permit development at once into a new individual but it is sufficient to permit some degree of independence in motor reaction, consequently, at some time when the worm is creeping the posterior end attaches itself and the rest of the body pulls away from it, as in *P. dorotocephala*. Apparently the greater part of the body in old fragmenting animals consists of a series of these small zooids for in most animals fragmentation continues until only the anterior third or fourth of the body together with the head remains. This anterior piece may then encyst or may undergo rejuvenescence without encystment and after some weeks give rise to a new posterior end, or in some cases it dies.

The posterior zooids are present only dynamically and not morphologically, at least not visibly, and they are not to be thought of as absolutely fixed stable entities. When the animal is strongly stimulated it is able to control the whole length of the body and for the time being the posterior zooids may almost or quite cease to exist, only to reappear after the stimulation is over. When such zooids are established the regions at their ends must be subjected to constantly varying correlative conditions. Sometimes they may form a physiological posterior part of one zooid, at other times an anterior part of another and at still others a part of neither. Such changes in correlative conditions must tend to weaken and eliminate the existing structure in those regions since the development of such structure depends on a certain degree of constancy in correlative factors. In this way zones of structural weakness arise and these are the zones where separation occurs.

Occasionally, either in consequence of weakness or perhaps because the physiological isolation of the posterior regions is insufficient the worm fails to fragment. In such cases parts of the body may become greatly elongated and a string of connected masses may arise. Figure 6 shows such a case. In the posterior region four distinct masses can be distinguished. These are connected by slender bands which are merely portions of the body greatly reduced in diameter. These four masses are connected with the anterior portion of the body by a long slender band resulting from the stretching of the middle region in consequence of the attempts of the head region to pull away from the attached posterior parts. These greatly elongated regions of the body consist of little more than the body-wall and muscles; the alimentary tract and the parenchyma may be almost or entirely squeezed out of them. This animal finally became surrounded by a cyst in the form shown in the figure, but later the connecting strands apparently atrophied, the pieces became entirely separate and each produced a whole worm.



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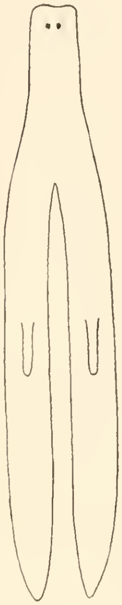
VI. The Development of the Whole Animal Within the Cyst.

The development of the animal from the encysted piece, whether isolated artificially by section or by the natural process of fragmentation, is similar in all respects to the regulatory development of pieces which reproduce new wholes without encystment. This is shown to be the case by the removal of the cysts from pieces at various stages of the process. In all cases the pieces are simply undergoing regulation. The process within the cyst may, however, be slower than in the unencysted piece, probably because the supply of oxygen within the cysts is less than in the water.

The natural method of asexual reproduction in this species does not then differ essentially in any way from the process of experimental reproduction. The process of fragmentation gives rise to the same conditions in the piece as experimental isolation by section and the further history is the same in both cases.



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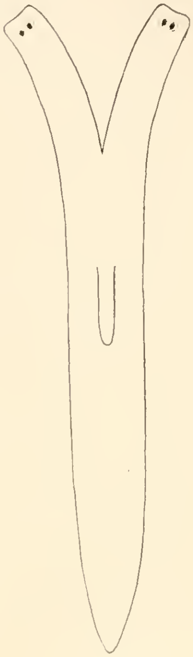

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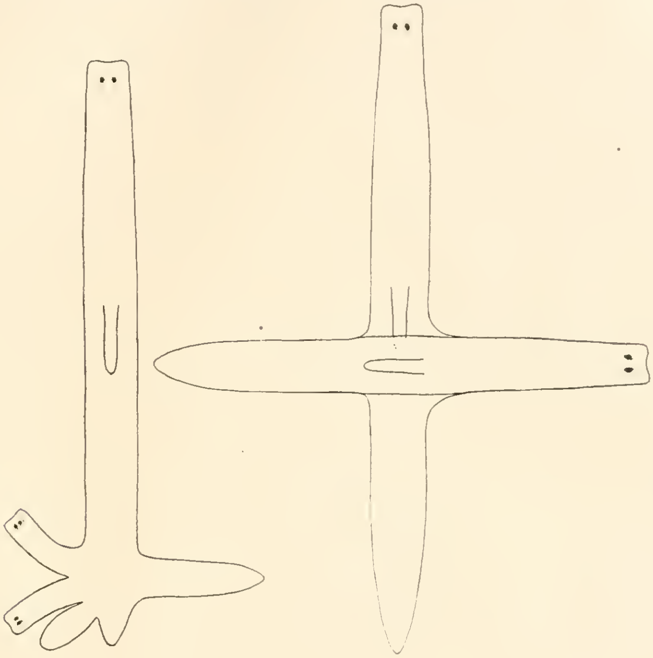
Many teratological forms result from irregularities in fragmentation or incomplete separation. The most common are partial duplications of anterior or posterior regions (Figs. 7 and 8) but various other forms appear. In Fig. 9, for example, a case is shown in which an incompletely separated posterior piece gave rise without encystment to two heads, a tail and two outgrowths of uncertain character, and Fig. 10 shows a case in which two worms with axes at right angles to each other are united by the middle regions of their dorsal surfaces. Ordinarily the larger animal carried the other about on its back as in the figure, the ventral surface of the smaller worm being uppermost. Fig. 11 represents a case of so-called axial heteromorphosis and in Fig. 12 two heads appear at the posterior end of the larger individual and dorsal to them a tail. Evidently new polarities arise very readily in the small pieces which result from fragmentation, probably because the pieces are so short that the original axial gradient (Child, '13*c*) is practically eliminated and chance differences in the rate of metabolism in different parts of the piece are sufficient to establish new polarities.



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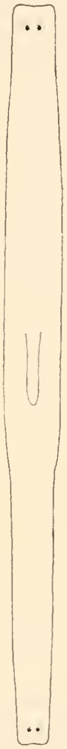
VII. Conclusion.

In *Planaria velata* the individual very evidently undergoes a process of senescence as it grows and either experimental or natural asexual reproduction brings about rejuvenescence. Moreover, the animal apparently returns to essentially the same physiological stage with each generation, for the species is able to persist without sexual reproduction and, as a following paper will show, numerous asexual generations have been bred in the laboratory without any indication of senescence of the stock.

I have shown elsewhere (Child, '11*b*) that the regulation of isolated pieces of *Planaria dorotocephala* brings about rejuvenescence to a greater or less extent, according to the size of the piece, the smaller piece giving rise to an animal which is physiologically younger than that produced by a larger piece. In that species starvation may also be a factor in rejuvenescence. Some experiments on the effect of starvation on *Planaria velata* will be described in another paper. At present it need only be said that the result is the same in both species.



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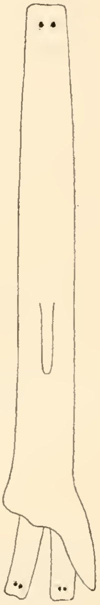

12

In my earlier paper on senescence the conclusion was reached that senescence results from the accumulation of structural products of metabolism which constitute in one way or another obstacles to the chemical reactions. The processes of differentiation and growth undoubtedly operate also in another way not considered in the earlier paper, to bring about a decrease in the rate of metabolism per unit of weight or volume. What we are accustomed to call the undifferentiated or embryonic cell represents the general metabolic substratum of the organism. Differentiation consists in the formation and accumulation of certain substances in the cell, some of which constitute more or less permanent structural features. At least certain of the substances composing these structural features are relatively stable under the usual physiological conditions and while certain chemical changes may occur in them, they are not broken down and eliminated to so great an extent as certain other substances. This relative stability must, in fact, be the basis of their persistence as elements of structure. The accumulation of these structural substances within the cell brings about a decrease in the general metabolic activity per unit of weight or volume because it decreases the proportion of the material involved in the general metabolic reactions to the inactive or less active material. The decrease in the proportion of the general metabolic substratum characteristic of the embryonic cell constitutes to some extent a histological criterion of the physiological change in the cell.

In short, the decrease in rate of metabolism per unit of substance, which is characteristic of development and senescence, is undoubtedly due in part to the fact that the proportion of the cell substance concerned in the general metabolic activity is decreasing and the proportion of less active or relatively stable substance is increasing. Changes in the size of the cell or in the size relations of nucleus and cytoplasm (Minot, '08) are not necessary factors in the result.

To what extent the decrease in the rate of metabolism during senescence is due in a given case to actual decrease in the rate of chemical reaction and how far to a decrease in the proportional amount of chemically active or more active substance is often difficult to determine, but it is probable that in some cases, or even in some cells of the individual, the one factor and in others the other is the more important.

As regards *Planaria velata*, the facts are that the rate of metabolism decreases during growth and development and increases when the substances previously accumulated are removed, either by regulatory reorganization, or by starvation. These facts show very clearly that in one way or another the accumulation of material in development decreases the rate of metabolism and its removal brings about an increase in rate. Senescence and rejuvenescence in this species consist essentially, I believe, in these changes.

Summary.

1. After a period of growth and activity *Planaria velata* undergoes fragmentation from the posterior end forward, the fragments encyst and give rise by a process of regulation to whole worms of small size.

2. During the period of growth the worms are undergoing senescence, as the decrease in rate of metabolism indicates, but the small worms which emerge from the cysts are physiologically, as well as morphologically young, possess a high rate of metabolism and are capable of repeating the life cycle.

3. In pieces isolated by section the frequency of encystment increases as the level of the piece becomes more posterior in the body, with decreasing size of the piece, with rising temperature and with increasing age of the animal. The facts indicate that encystment is the result of stimulation. The stimulation may result from section, from fragmentation, from a rise in temperature or from other conditions.

4. The development of the encysted piece into a new whole animal is essentially the same process as the regulatory development of unencysted pieces.

5. This species is able to live for an indefinite number of generations without sexual reproduction. Each new asexual generation represents a return to essentially the same physiological and morphological stage. In other words, senescence leads to reproduction and the process of rejuvenescence in each asexual cycle carries the organism back to the same stage of youth.

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DEVELOPMENTAL CHANGES OF PIECES OF FROG EMBRYOS CULTIVATED IN LYMPH.

S. J. HOLMES.

In the course of some experiments on the behavior of the epithelial tissue of frog embryos when cultivated in lymph or plasma it was found that pieces of tissue sometimes underwent developmental changes. During the comparatively short time in which material was available for experimental work, opportunity was not found for devoting as much attention to the changes in these pieces as was desirable. Nevertheless a few of the things observed were sufficiently striking and suggestive to be worthy of record, and it is hoped that the observations may be made more complete at a future date.

The material used consisted of embryos and young larvæ of *Rana* taken out of the jelly a short time before they were ready to make their natural exit. The jelly was placed for a few minutes in an antiseptic solution, and the embryos were afterward cut to pieces in sterile Ringer's solution. Each piece was then mounted in a hanging drop of lymph or plasma of the adult frog, sealed up in a hollow slide, and kept in a cool place. Every few days the piece was transferred to a fresh medium.

Changes in the ectodermic epithelium were quite manifest a few hours after the pieces were mounted, and in one or two days remarkable strands and sheets of ectodermic cells were to be seen extending into the culture medium. These structures and the behavior of their component cells are more fully described in another paper now in course of publication. But aside from the movements of the ectoderm, there were, in certain of the pieces, marked developmental changes of the internal parts. Irregular fragments of tissue frequently became more rounded in form; sometimes they put out lobes or processes of various kinds, and, in some cases, they increased noticeably in size. In a piece from the head region of a late embryo shortly before the rudiments of gills made their appearance, there were developed, in addition to several larger lobes, a number of finger-like processes which very closely resembled the filaments of the gills. The central part of these projections was composed of connective tissue, and the epithelial covering consisted of a layer of columnar ciliated cells. These outgrowths began three days after the preparation was made, and they were practically completely formed in a week. Fifteen days after the piece was mounted it had assumed the form shown in Fig. 1. The cilia on the projections were beating, and in fact they continued active for two more weeks, but there was little further change in the general form of the piece. When originally mounted the piece was opaque, and the cells composing it contained a large amount of yolk. After two weeks it had become nearly transparent; the larger part of the yolk in the cells had been assimilated, and a typical connective tissue had developed in the interior from the comparatively unspecialized cells of the mesoderm. The piece was covered completely by ectoderm which, over most of the surface, was flattened, but assumed a cylindrical form on the more prominent projections. In the third week a layer of epithelium was sloughed off from a large part of the surface of the piece.

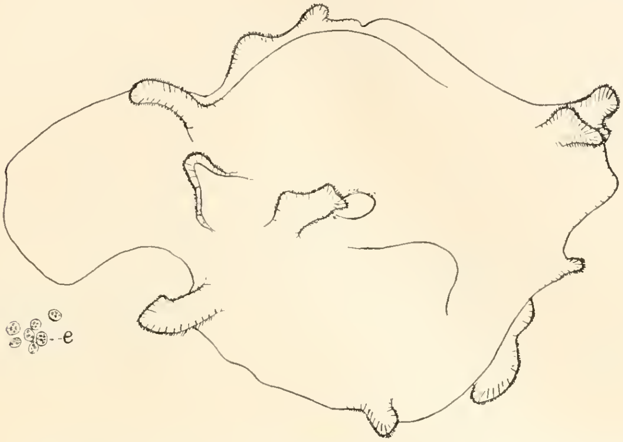

FIG. 1. Piece from the head of a frog embryo drawn fifteen days after being cultivated in lymph. *e*, isolated ectoderm cells.

Whether the finger-like processes represent gill filaments is more or less open to question. Processes of this kind failed to make their appearance from the hinder part of the embryos.

Should they be gill filaments they would afford a case of the self differentiation of an organ in the absence of certain stimuli, such as those afforded by the blood supply, which normally are associated with their formation.

Fig. 2 represents a development from a cross section of a late embryo near the middle of the tail region. This piece, like the one just described, was at first opaque, owing to the large amount of yolk in the cells. A week later it became quite transparent, and numerous pigment cells became differentiated beneath the ectoderm. Externally it was completely covered by a layer of flattened epithelial cells. The connective tissue within the piece had become more specialized. At either end of the piece there appeared a prominent outgrowth, the one at the posterior end curving upward and forward. There were two large cavities lined with a single layer of much flattened endothelial cells. These cavities were much larger than any spaces in the original piece of the embryo, and not improbably represent closed and distended blood vessels. The notochord showed at each end an extension of cells which suggested that the organ was undergoing regeneration in both directions. These extensions lay entirely within the new outgrowths from the cut ends of the piece.

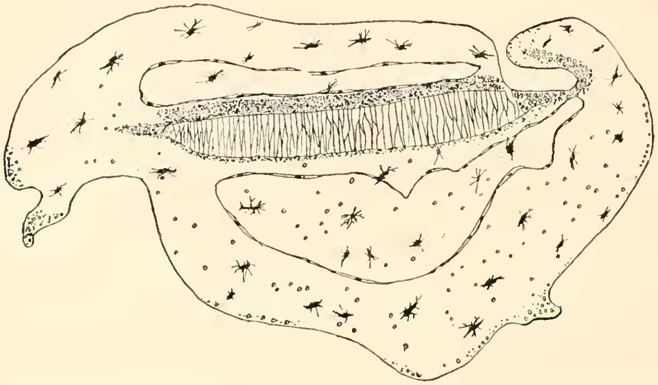

Fig. 2. A cross section from near the middle of the tail of a frog embryo drawn seven days after being cultivated in lymph.

Fig. 3 represents a developed fragment taken five days previously from near the middle of an embryo. This piece contained some entoderm which is included in the dark pigmented mass near the center. At one side there was a thin vesicle filled with fluid. The ectoderm cells covering this vesicle were much flattened. Ten days later the whole piece was larger, owing to the increase in the size of the hollow vesicle which had come to surround most of the rest of the fragment (Fig. 4). The pigmented mass and the tissue around it had assumed a more nearly spherical form, and the whole structure had become more nearly spherical also. Apparently the chief factor in the form changes in this case was the absorption of fluid which caused the increasing distension of the thin walled vesicle. A similar process may account for the large spaces in the fragment shown in Fig. 2. In transferring the nearly spherical fragment to fresh lymph the thin wall of the vesicle partly collapsed, but it was subsequently distended to its previous form. During the time the piece was kept alive, which was about five weeks, it showed no other marked changes in form.

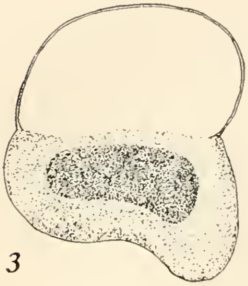

FIG. 3.

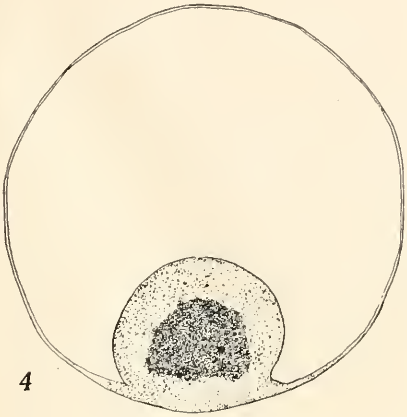

FIG. 4.

FIG. 3. A piece which developed from a fragment of a middle portion of a frog embryo.

FIG. 4. The same piece as is shown in figure 3, but drawn ten days later.

UNIVERSITY OF CALIFORNIA,
June 16, 1913.

NEMATOLAMPAS, A REMARKABLE NEW CEPHALOPOD FROM THE SOUTH PACIFIC.

S. STILLMAN BERRY,

Redlands, California.

In a collection of Cephalopoda from Sunday Island, one of the Kermadec Group, which has been recently placed in my hands for investigation, is a new oegopsid possessing features of such general interest that the sender of the specimens, Mr. W. R. Oliver of Auckland, has kindly permitted me to offer the following brief preliminary account of the species in an American publication pending the appearance of a full report on the collection in the Transactions of the New Zealand Institute.

Nematolampas regalis new genus and species.

The body is small, cylindro-conical in outline, and terminates in a not very sharp point posteriorly. The fins are very large in proportion to the body, their combined width being as great as the length of the latter, although they are not quite half so long. They are practically continuous with one another above, at least posteriorly, and their union with the mantle is very insecure in alcoholic material. On each side of the extreme posterior tip of the body and lodged in the angle between the fin and mantle is a small swelling containing a conspicuous heavily pigmented body of spherical outline which from its general appearance has undoubtedly a photogenic function. This entire mass with its contained organ is not very firmly attached and becomes readily dislodged when the fins have been loosened.

The head is relatively large, short, and rounded. The eyes are very large. Bordering the ventral periphery of each eyeball is a longitudinal series of five beadlike photophores of a reddish color. Of these the central one is considerably the largest and so conspicuous that it is readily visible through the outer integument. Both it and the smaller organs just adjacent to it are circular-ovate in outline, but the terminal organs are comparatively narrow and elongate.

The arms are unequal in length, and in one instance, that of the ventro-lateral pair, this inequality is of a very extraordinary nature. This arm pair is in any case the longest and their basal portions much the stoutest, but furthermore each terminates in an exceedingly slender beaded filament which when straightened out is considerably longer than the entire remaining portion of the animal and is devoid of suckers, though the arm proper is normal in this respect. The entire arm bears a succession of small but heavily pigmented photogenic organs scattered at various intervals along its outer margin. On the filament these appear as swellings or tubercles, often half as large in diameter as the filament itself, but on the basal portion of the arm they become rather deeply imbedded and are not easily seen except by transmitted light. Including its filament the better preserved arm of this pair carries a total of thirty-one photophores. The remaining arms are normal as regards their extremities, but those of the dorsal and dorso-lateral pairs bear each a single photophore near the tip. All the arms bear two rows of small suckers, but no hooks. The order of relative length is 3, 2, 4, 1.

The tentacles are about as long as the mantle, their clubs little expanded and armed with four rows of suckers. Each has a pale indistinct swelling in the stalk a short distance from the base and a similar one a little distance below the club. Except that the proximal one is situated considerably nearer the base of the stalk, these swellings occupy a region analogous to the position of the tentacular photophores described by Chun for *Thaumatolampas* (1903, p. 570, fig.; 1910, p. 59, pl. 1–4) and quite likely represent similar structures.

As in *Thaumatolampas* also some of the more important luminous organs lie within the mantle cavity and in the living animal are visible only by reason of the transparency of the pallial tissues. These organs are eight in number and throughout are clearly homologous in the two genera. The two anterior are situated one on either side of the alimentary canal just back of the funnel and correspond to the anal organs of Chun.

In the present form they are quite small. A little behind the middle of the body is a very conspicuous transverse series of five organs. The central one is unpaired. Close to it on each

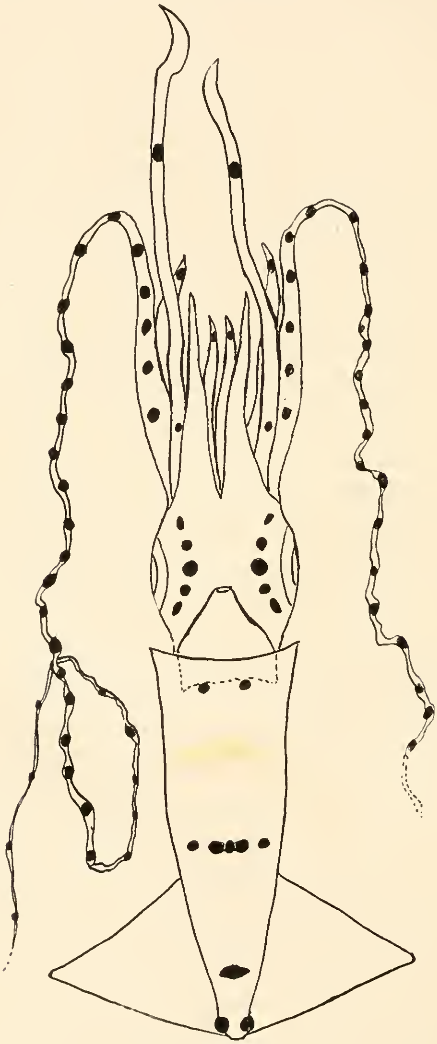

FIG. 1.

side is a very large flattened organ. The terminal organs are the smallest of the series and situated one at the base of each gill. In the posterior part of the pallial chamber between the fins is a large unpaired organ.

The medio-dorsal length of the mantle is 32 mm., the width of the same 11 mm. The length of the entire animal exclusive of the tentacles and the filaments of the third arms is about 57 mm. The length of the third arm pair is over 70 mm.

From the above account it will be seen that this small squid possesses no fewer than ninety definitely and symmetrically arranged photogenic organs, and it may well be that there are even others which have escaped my search, as the opacity of the tissues in preserved material in many cases renders their detection difficult. Those which I have been able to make out are shown somewhat diagrammatically in the accompanying drawing, and also in the following tabular statement:

Ventral periphery of eyeball	10
Tip of dorsal arms	2
Tip of dorso-lateral arms	2
Ventro-lateral arms	62+
Tentacles	4
Within pallial chamber:	
Anal	2
Branchial	2
Abdominal	4
Posterior extremity of body	2
Total	90

No cephalopod heretofore described is at all comparable to this creature excepting the wonderful *Thaumatolampas diadema* Chun, already mentioned, and the *Lycoteuthis jattai* of Pfeffer ('00, p. 161), both of which are now thought by the latter author to be based upon the same species ('08, p. 294). Chun, in the course of his amazing description of the luminous organs of *T. diadema*, says, "Unter allem, was uns die Tiefseetiere an wundervollen Färbungen darbieten, lässt sich nichts auch nur annähernd mit dem fast magischen Kolorit dieser Organe vergleichen. . . . Es war eine Pracht!" But to judge merely from the anatomy of *Nematolampas*, even *Thaumatolampas* must be outmarveled in life by this wonderful mollusk from the Kermadecs.

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BIOLOGICAL BULLETIN

A STUDY OF THE EFFECTS OF INJURY UPON THE FERTILIZING POWER OF SPERM.

NEIL S. DUNGAY.

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I. INTRODUCTION.

This paper deals with the results obtained by fertilizing normal eggs with spermatozoa injured by various methods. The results have a bearing upon some of the problems of fertilization.

They also throw light upon the theories dealing with the production of defectives by the action of injurious agencies upon the germ cells previous to the time of fertilization.

The experimental work was done at the Marine Biological Laboratory at Woods Hole, Mass., during the summers of 1911 and 1912. The results given are based upon observations made upon more than 600 cultures of living *Nereis* eggs and 200 cultures of *Arbacia* eggs. Samples from the various cultures were preserved for later cytological study, the results of which are also reported in this paper.

II. Materials and General Methods.

A. Occurrence.

In the evening from the time of darkening until about ten o'clock, sexually mature males and females of *Nereis limbata* swim freely at the surface of the Eel Pond at Woods Hole. Although they are most easily collected during the two weeks following the full moon, it is possible to find a few nearly every evening of the lunar month. The breeding habits have recently been described (Lillie and Just, '13) in a very interesting paper which gives in considerable detail the methods of catching and keeping the worms.

B. Collecting.

For my experiments the animals were caught in nets by lantern light, placed in separate finger bowls of sea water, and taken to the laboratory buildings at about 9:30 P.M. The worms were then washed in running sea water and the finger bowls were cleansed and refilled, covered with glass plates and placed in running water to keep cool. Early the next morning the accumulations of mucus were removed and the water was again changed. When carefully cared for in this manner, very few of the worms shed their germ cells during the night. The males practically never shed their sperm cells under these conditions. During one month in which a record was kept, over 90 per cent. of the females captured retained their eggs. By this method I have sometimes kept the females without shedding for as long as 60 hours, though, for experimental purposes, the eggs which were retained so long were not used. The experimental work was usually started as early in the morning as possible so as to use the material in a fresh condition. A few experiments were carried out shortly after removing the animals from the Eel Pond. Since no better results were secured by working at night, the material was usually kept until morning when conditions for work were better.

C. Removal of Germ Cells.

The abundant eggs and sperm were removed by snipping the sides of the body wall at intervals, with scissors. If no water is used the sperms push out as a white mass nearly free from liquid and the eggs may be secured as a thick greenish mass. As a rule, however, the operation was conducted under water. In all cases the usual precautions were taken to avoid the introduction of stray sperm cells. The worms were thoroughly washed in a jet of salt water and, in the earlier experiments, in boiled salt water as well, a precaution which I afterwards found to be unnecessary since the sea water from the laboratory supply was repeatedly tested and found to contain no active *Nereis* sperm. The bodies of the animals used were quickly removed from the dishes of sperm and eggs so as to avoid, as far as possible, any contamination from body fluids, and every precaution was taken to avoid abnormalities due to outside influences such as strong sunlight, bacterial action, mechanical agitation, etc.

D. Culture Methods.

An effort was made to use a minimal quantity of sperm for the process of insemination, in order to avoid, as far as possible, errors due to polyspermy. In the case of the injured sperm, a large quantity was often necessary to secure a small percentage of fertilized eggs. Usually a small number of eggs was inseminated first in a small quantity of water to which a liberal supply of water was soon added. In this manner I was able to secure a sufficient number of fertilized eggs to make preservation possible. Up to the time when the larvæ begin to swim, the water was changed several times. After this period it is very difficult to renew the water without losing many of the larvæ. An effort was made to keep from crowding the developing eggs since this seems to be a source of abnormality. Any error which might creep in here was checked by the controls since they always contained at least as many eggs as were kept in the experimental cultures.

E. Controls.

Along with each experiment two or more control cultures were kept. One control consisted of a dish of sea water containing a part of the eggs which were not inseminated. The other contained about the same number of eggs as was kept in each of the experimental dishes, inseminated with injured sperm; these were as closely followed during the period of observation as were the experimental cultures. In all experiments in which the sperm cells were subjected to the action of reagents it is impossible to avoid carrying over a trace of the reagent used, to the dishes of eggs. Control experiments were made in such cases by adding to the eggs at the time of insemination, as much of the reagent as was introduced into the experimental cultures. In nearly all cases the eggs and sperm used in the controls came from the same animals which furnished the materials for the experiments.

F. Treatment of Sperm Cells.

Attempts to injure the sperm cells as much as possible without preventing their attachment to the eggs proved unsuccessful in many cases. Either the sperm were killed or they were not sufficiently affected to give striking results. With improvement of the technique consistent results were secured, although these results indicated a great variation in the intensity of the injuries to the sperm cells, thus rendering it impossible to repeat an experiment with a complete duplication of results.

The sperm cells were injured in various manners. Inasmuch as some methods gave promise of easier and more striking results or seemed less open to criticism, but few experiments were made along other lines in order to give more time to follow out the more desirable methods. Injury was attempted by soaking the sperm cells in distilled water or in diluted sea water; by treating them with weak alcohol; by exposing them to the action of dilute acids or alkalis; by freezing; by heating; and by keeping them so long that they were nearly dead. Since it proved to be so difficult to injure the sperm without destroying their motility it seemed to be desirable to try the effects of the X-rays or of the radium emanations; but as the proper appliances were not procurable at the time, I was unable to carry out this part of the work. An attempt was made to get results by using sunlight but the sperm cells gather together in such a way that they protect each other. No attempt was made to carry this method far although it might prove useful with proper technique.

G. Cytological Methods.

For later cytological study of the early stages, a large number of samples were taken from the experimental cultures and fixed in Meves's modification of Flemming's fluid, made as follows: 3.5 c.c. osmic acid, 2 per cent.; 15 c.c. chromic acid, 0.5 per cent.; 3 drops glacial acetic acid. The eggs were exposed to the action of this solution for about 40 minutes. Iron hæmatoxylin was used as a stain. The larval stages were preserved either in Gilson's mercuro-nitrate fixing fluid or in Bouin's mixture of formalin and picric acid.

The fact should be mentioned at this point that it was found to be impossible for one person to make as careful observations and notes upon the living material, when preserving a series for later study, as were made when doing nothing else except following the developing eggs under the microscope. As a consequence the preserved samples are of two kinds, viz.: (1) a long close series with as much recorded data as the time allowed, and (2) irregular preservations made during extended periods of observation. Usually in getting a long series fixed it was necessary, especially in the earlier stages, to run at least two series at one time, since it is impossible to tell with certainty just how much the sperm cells used for inseminating have been injured. Again it should be noted that in the preservation of a long series, those samples first fixed often contain a greater proportion of the more normal eggs than those preserved later. This is due to the fact that the most normal sperm cells seem to initiate development earlier than do the others. Since the eggs which have formed jelly are more readily picked up by the pipette, they will tend to be taken from the cultures in greater proportions in the earlier part of the preserved series. An effort was made to overcome this by using several parallel cultures in different dishes but without entire success since the number of eggs from a single female is limited and since it does not seem wise to use eggs from two or more females, in the same experiment.

III. Outline of Development of Nereis Eggs.

Although the maturation, fertilization, and development of the egg of *Nereis* have been described in more or less detail by others (*e. g.*, Wilson, '92, Lillie, F. R., '11, and '12) it seems desirable to review a few of the principal points at this time. The eggs, which vary considerably in size, are about 100 microns in diameter. Their irregular shape at the time of laying is soon lost and they take the form of a sphere slightly compressed in a polar direction. They are of a greenish color and moderately transparent. A polar view, which is the one usually obtained, shows the large germinal vesicle in the center of the egg and an imperfect double belt of large oil drops, numbering approximately from ten to twenty. Just beneath the vitelline membrane is a radially striated cortical layer which breaks down immediately following insemination, throwing off a thick envelope of jelly and leaving a conspicuous perivitelline space. The jelly is readily demonstrated by the use of India ink ground up in sea water, although it is nearly invisible in pure sea water. Since the unfertilized eggs normally lie in contact at the bottom of a dish and, upon insemination, become separated by the jelly, it is easy for the naked eye to determine whether or not a large part of the eggs have started to develop. A microscope, however, is necessary to determine the condition of any individual egg. As a general rule unfertilized eggs do not form jelly upon standing undisturbed, at least not for several hours. Yet close examination usually reveals a mere trace of eggs which have formed this secretion. Inasmuch as strong mechanical agitation will induce jelly formation this is perhaps due to violent contact with the scissors upon opening the female, or possibly there may be a few eggs which are extraordinarily sensitive to stimulation. The latter view is rendered probable by the fact that the proportion of eggs with jelly varies considerably among different lots, so much so that an occasional batch of eggs is found in which the majority form jelly. This jelly formation is probably not due to accidental insemination since fertilization cones are not seen and cleavage does not result. Yet to guard against possible misinterpretation an unfertilized control was kept for each experiment and examined along with the experimental materials.

The active spermatozoön may be seen to attach itself to the egg. It immediately becomes quiescent and remains motionless until it is drawn into the egg. The outflow of jelly, which continues for some fifteen minutes after insemination, now sweeps away all superfluous sperm cells, which have in the meantime lost their power of motion. Just beneath the attached sperm cell an attraction cone is pushed up by the egg cytoplasm until it unites with the membrane. It then gradually disappears, carrying with it the membrane so as to form a depression in which the sperm is now concealed. This all happens during the first 25 minutes after insemination. In the meantime the germinal vesicle has disappeared and the egg has assumed an irregular shape. About 45 minutes after insemination the egg resumes its spherical form and soon the sperm, after again coming prominently into view for a short time, is drawn into the egg. Shortly before the close of the first hour succeeding insemination the first polar body appears and is followed in a few minutes by the second. The polar bodies are easily seen at this time against the now yolk-free region of the pole. In a profile view they are very conspicuous. In the course of the second hour the first two planes of cleavage appear. The first plane, as has been stated by Just ('12), passes through the point of entrance of the sperm, dividing the egg unequally. The development proceeds rapidly from this point. Movement is often seen in twelve hours and fine trochophores are formed by the end of the first day. In the second day the animal begins to elongate and the larval setæ grow out to a relatively great length. Naturally the time element, as given above, varies greatly with the temperature.

IV. EXPERIMENTS.

The sexual products of both *Nereis* and *Arbacia* were utilized for experimental purposes. However at the time when I could use *Arbacia*, the control cultures did not run as well as might be desired and so *Nereis* material was used for the greater part of the work.

A. Upon Nereis.

In the earlier experiments upon *Nereis* I subjected the sperm cells for a certain time to the action of graded strengths of various reagents. Later I found that the most satisfactory procedure is to keep the sperm cells, after removal from the body of the male, until they are nearly dead or to keep them for a few minutes at a temperature of about 44° C. The latter method seems to be less open to criticism since no substances of a possibly toxic action are transferred to the egg cultures by the process of insemination.

All of the methods of experimentation which were used give essentially similar results, indicating that the action upon the sperm cells is probably not specific in any case. It seems rather that there is produced a general decrease in vitality which may manifest itself by a retarded development, by a high mortality or by the production of forms which are more or less abnormal in structure or behavior.

1. *Heat.*—Perhaps the most uniform results in the whole series of experiments which I have carried out were obtained through the exposure of the sperm cells to a certain degree of heat. This was accomplished by mixing the sperm cells with sea water, which was then placed in the bottom of a test tube, care being taken to prevent any of the liquid from touching the walls of the upper part of the tube. A thermometer was then introduced into the bottom of the test tube and was used to stir the liquid and to take the temperature readings every minute. The test tube was nearly immersed in a large beaker of warm water. The temperature was maintained in the bath thus formed, by means of an alcohol lamp or by adding to the bath warmer water from another container. In this manner it is possible to keep the temperature under reasonable control.

Particular care was taken to insure the perfect cleanliness of all pipettes. None of the liquid of the test tube was allowed to splash upon the sides of the tube so as to be above the level of the water in the bath. A large number of dishes of eggs were made ready and one or more of these were inseminated every minute until either all the sperms had been used up or the dishes of eggs had all been inseminated. In this way there were usually obtained one or more dishes of eggs which were inseminated at the critical time which occurred from 7 to 20 minutes after the sperm cells were placed in the warm bath. The variation in time is probably due to fluctuations in the temperature of the bath, small though they may be. This is borne out by some experiments in which slightly higher temperatures were used and in which the critical time came much earlier, so early in fact that it was difficult to catch the sperm cells at just the proper stage for use.

Since it proved to be very difficult to determine in advance which culture would be the best for study, the preservation of a long series from the best cultures is largely a matter of chance. But by preserving a large number of series for cytological examination, a few have been obtained which are satisfactory for study. The others, although they show essentially the same features, contain so many eggs which develop normally that the study of the affected eggs is very time consuming.

In all, 23 experiments, involving several hundreds of cultures, were made by this method. Since the earlier work was carried out in a different manner, which gave me records of the course of development of the experimental cultures but no continuous series of preserved samples, the primary purpose of this set of experiments was to obtain material for cytological work. Accordingly the records of these experiments are not as full as might be desired in the case of any one experiment.

To illustrate the method of procedure the record of experiment 65 of July 4, 1912 is given. The eggs were removed from the female at 1:39 P.M. Sperm cells removed from male at 1:40 P.M. Sperm placed in bath at 1:47 P.M. Temperature of bath at end of successive minutes (read from centigrade thermometer in test tube) 43°; 44°; 43.4°; 43°; 44°; 44°; 43.8°; 43.3°;

43°; 43.7°; 43°; 43.7°; 43°; no later records. Samples of eggs from one female, numbered 65.1, 65.2, 65.3, 65.4, and 65.5, were inseminated with heated sperm at the end of 11, 12, 13, 15, and 16 minutes respectively. In the first four about 95 per cent. of the eggs formed jelly immediately. Sample 65.5 gave about 80 per cent. with jelly. All of the above were discontinued. 65.6 was inseminated at 2:04 P.M. with sperm which had been heated for 17 minutes. Jelly formation began at once but took place in a gradual manner, some eggs failing to give off jelly until the end of half an hour. Eventually about 60 per cent. formed jelly. Of the eggs with jelly 50 per cent. failed to segment, 15 per cent died before gastrulation, 25 per cent. formed trochophore larvæ later than did the controls, and 10 per cent. behaved in a seemingly normal fashion. Discontinued before the formation of the larval setæ because all had been preserved. Control of infertile eggs showed only a trace of jelly formation after 18 hours. The inseminated control developed normally. A series of preservations from the experimental culture 65.6 was made at 10 minute intervals, beginning at 2:24 P.M.

Experiment 63, July 3, 1912, was conducted in a similar manner and furnishes a somewhat fuller record of the later stages. 63.5 was inseminated at 10:36 with sperm which had been kept in a warm bath (about 44° C.) for 14 minutes. Over 70 per cent. formed jelly. First cleavage was observed at 12:15 P.M., 7 minutes later than in the case of the control series. At 1:25 P.M. the control culture was nearly two cleavages ahead of the experimental culture. About 5 per cent. of the eggs in the experimental culture failed to segment after forming jelly. At 8:45 A.M. on the following day the experimental culture was very much less active than the control. About 5 per cent. had died in cleavage stages. 49 hours after insemination the control animals were in excellent condition and were sending out the second set of larval setæ. The experimental culture showed clearly that the injury to the sperm cells may cause abnormalities in those eggs which seem to behave normally in the earlier stages. 50 per cent. of the developing eggs had not gone beyond a trochophore stage. Most of these died soon, though a few continued to live for at least another day without any change except an increase of size. This growth is probably due to an increase in water content and gives the animals a dropsical appearance. 25 per cent. were as well developed as the control animals. The remaining 25 per cent. were in various stages from the trochophore to the formation of the second set of larval setæ. Some of them seemed to be normal in every way except that their development was retarded. Others were less active. A small number, perhaps 5 per cent., seemed to be as far along as the controls but had no setæ or were deficient in some other way. Most of these died within a few hours. The controls developed in a normal manner. The uninseminated controls showed no development beyond the formation of jelly in about 0.3 per cent. In all the cultures of the above experiment the conditions were made as good as possible by changing the water, removing dead eggs, etc.

Without attempting to give other definite records, I may summarize briefly the different types of effect observed in the series of experiments using heat as a means of altering the sperm cells.

(*a*) In some cases all of the jelly did not form and the peripheral alveoli of the egg were not entirely emptied. This occurred in from a trace to nearly 10 per cent. of the eggs in the experimental cultures. Some eggs formed a mere trace of jelly, some formed about half of the normal quantity, and some had nearly as much as normal. The different cases observed gave the impression that a larger stimulus caused a greater jelly formation. It seemed as though a sperm might in some way give only enough impulse to cause a part of the alveoli to be emptied, possibly due to a brief attachment to the egg. However no such egg was ever followed long enough to determine the cause of this partial jelly formation.

(*b*) After the jelly is completely extruded and the polar bodies have been formed, cells are commonly found in which no further development takes place. A few eggs do this in many uninseminated controls but there is no comparison in the frequency with which they may be found. In some experiments special care was taken to avoid mechanical agitation since violent mechanical stimuli may produce this effect. Yet the same results were found, indicating that maturation and jelly formation had been initiated by the sperm in some way, although later development failed to take place. In some experiments this has been seen in more than half of the eggs which formed jelly. All experiments which gave any indication of abnormality contained at least a few of this type of eggs. In those cultures in which these eggs were present in considerable numbers I failed to see as many fertilization cones as usual. This suggests either that the eggs were infertile, due to the failure of the sperm to enter, or that the development was carried this far in response to some unknown stimulus given at the time of insemination. Cytological examination is necessary to determine this point.

(*c*) Cytological examination of stained sections showed cases in which polar bodies appeared without jelly formation. No indication of this was seen in the living material as it was not supposed that it was possible. It would, of course, be difficult to find, even if such cases were known to occur.

(*d*) Development sometimes stopped after the first cleavage or more rarely after the second. This occurred in less than 2 per cent. usually. It was usually associated with

(*e*) an unsuccessful attempt to form a first cleavage plane. The behavior of such cases will be described under another heading in more detail.

(*f*) In some cases, as many as 0.5 per cent. in one experiment, the first cleavage plane divided the cell into two equal parts. Such cases did not usually divide again.

(*g*) In a very few egg cells the cleavage was uneven, forming 3, 5, or 7 cell stages which usually died without developing far. It is well known that uninseminated eggs may undergo a kind of pseudo-cleavage if allowed to stand for some hours but the cases mentioned here are not to be confused with this for several reasons. Their appearance is different. These forms appear much earlier than they ever do in the unfertile eggs. The control of unfertilized eggs never showed such pictures until much later and then they were of a different appearance.

(*h*) Other irregular forms of cleavage were seen, but very rarely. Since the later cleavage stages are more complex in appearance it is more difficult to find irregularities and they may be of more frequent appearance than my notes indicate. Again it may be possible that such cases are sometimes present in eggs fertilized with normal sperm. I have no reason to believe that my control cultures contained such cases.

(*i*) The rate of development in the experimental cultures often proved to be very irregular and was usually slower than that of the controls. For example, when the controls first have definite setæ the experimental cultures have none. Segments are marked out earlier in the controls. Early cleavage planes are often delayed, but this may be due in some part to the failure of the weakened sperm to reach the egg as soon as it should. But it seems certain that, even allowing for this fact, the early stages are somewhat retarded in development.

(*j*) In practically every experiment the controls lived longer than the experimental cultures and the death rate in them was not so much as one tenth as large. Although an effort was made to keep the controls under exactly the same conditions as the others there were usually fewer larvae in the experimental dishes (presumably a more favorable condition). Old eggs and organic materials were removed so as to eliminate any possible toxic action of products of decay. In some cases the water was changed from day to day but with little effect. It seems that the normal animals in the controls are able to withstand the artificial conditions incident to life in the laboratory for a much longer time than those in the experimental cultures, which are so weakened by the injury to the part which comes from the male parent, that they cannot exist under such conditions as are provided.

(*k*) The trochophore stage seems to be a difficult one to pass. It frequently happens that this stage is permanent. In some cases at about the time when segments should be marked out and setæ should appear, the animal becomes irregular in form or swells up and becomes transparent, due probably to the absorption of water. In some cases the trochophore remains in its original condition without change. In most cases death takes place soon after elongation should take place but I have often kept the permanent trochophores for a day or more after they should have elongated.

(*l*) When the larval setæ should form, it often happens that none or only a few of them appear. In some cases this was true of more than half of the larvæ. Very few experimental cultures showed less than 5 per cent. of such forms. There may be all grades of conditions from no setæ, through a few scattering ones, weak ones, and abnormally directed ones, to nearly perfect forms. In cultures inseminated with normal sperm, very adverse external conditions may produce this effect or something, in a small number of the larvæ, very much like it. But under the very best conditions that could be maintained this was always found in the experimental material, though never in the controls. Associated with this condition was the partial or complete lack of sensory appendages.

(*m*) The body form is sometimes altered. It may be permanently bent toward one side by the asymmetrical development of a single segment. Often the body is covered with small wart like projections, giving it a peculiar roughened appearance.

(*n*) The experimental cultures nearly always show a lack of vitality, as is evidenced by a lessened activity. The normal cultures are very active after reaching the motile stages. The others, at least in part, are usually more or less quiescent. The phototropic responses are much less uniform in the experimental cultures and do not take place so quickly.

(*o*) In addition to the above list of abnormalities there is always present, unless the unfertile eggs are removed from the cultures, a number of other types which are confusing at first. As it is not always possible to remove every unfertilized egg during the first few hours, one is likely to find a variety of things which are easily misinterpreted unless they are very carefully studied. Unless such things have crept into my counts through an occasional oversight no consideration will be given them.

Nearly all of the above list of abnormalities were present in most of the experimental cultures if they were not discontinued too early. The controls rarely produced any of them. The proportions of the different types naturally differed widely in the various experiments. If the sperm cells were injured to the limit, or nearly so, the abnormalities were largely confined to the early stages and death occurred before the later ones could appear, though a few individuals nearly always succeeded in reaching the later stages. If the sperm cells were not injured so much, the earlier stages were comparatively free from abnormalities and those of the later stages appeared, chiefly the permanent trochophores, the malformed bodies and the lack of setæ.

In these experiments, as in those to be described later, the number of eggs fertilized is inversely proportional to the strength and duration of action of the injurious agent, provided the proportions of eggs and sperm are constant. The degree of abnormality and the per cent. of abnormal forms is directly proportional to the strength and duration of action of the agent used. These statements, of course, are not to be taken as more than an approximation. No effort has been made to reduce the results to the form of an exact law. However the results of the series are consistent and conform nicely to the above generalizations.

2. *Delay.*—Striking results were obtained by keeping the sperm cells for some time before they were used for insemination. Naturally the extreme time which may elapse before the sperm cells become incapable of influencing the eggs varies greatly with the temperature, with bacterial conditions, and, I suspect, with the general vitality of the cells. The complexity of the conditions makes it extremely difficult to completely duplicate the results of any particular experiment. Oftentimes it happens that the results in experimental cultures are nearly normal and again development may not appear at all. Yet the results all seem to be consistent, the differences being in degree only. As in the set of experiments described under the head of heat, the abnormal types are undoubtedly due to certain degrees of injury to the sperm cells. The possibility of the results being due to toxic substances produced by bacterial action or sperm metabolism during the period of delay, and introduced into the egg cultures at the time of insemination, seems to be rather remote. In order to minimize any such effect, the water was changed on the egg cultures several times shortly after insemination.

One of the several extreme cases of effect upon early development is found in experiment 34 of July 15, 1911. The uninseminated control remained unchanged. The fertilized control was inseminated at 2:11 P.M. At 3:15 P.M. the first cleavage was practically completed and many eggs were dividing the second time. At 3:25 the majority of the eggs were in a 4 cell stage. At 9:00 A.M. of the following day all were normal trochophores. The second day brought forth setæ and succeeding days showed only a normal development. 34.1 consisted of fresh eggs and a large quantity of sperm which had been kept in a corked vial for 21 hours at room temperature. The sperm and eggs were mixed at 1:35 P.M. Less than 5 per cent. of the eggs formed jelly. At 2:59 P.M. there were many attempts to form a two cell stage. It will be noticed that this is over 20 minutes longer after insemination than the time at which the controls were nearly through with the first cleavage. Very few normal 2 cell stages resulted. Usually the constriction appeared in the proper location but a few cases were observed in which the attempted plane of cleavage would have divided the cell into equal parts, had it been completed. In the great majority of cases the constriction lasted for a few minutes and then began slowly to disappear, the cell becoming irregular in outline. For example one cell was observed to be in the early process of first cleavage at 3:18 P.M. Slight progress was made for a minute and then no change took place until 3:23 P.M. when the furrow began to disappear. At 3:37 P.M. the furrow was invisible and there was no trace of the attempted cleavage. Irregularities in cleavage were also seen. At 3:55 P.M. a 5-cell stage was noted. A 4-cell stage with two very large cells diametrically opposite to each other was observed at the same time. Another distinct 5-cell stage was seen. Another 4-cell stage, as seen from a polar view, had cells of equal size, the polar bodies appearing to be normal. At 3:40 P.M. and following, another case of attempted cleavage, similar to the one described above, was seen. At 3:44 P.M. a 3-cell stage was found. Many one cell stages which had formed jelly and thrown off polar bodies were now evident. It was not determined whether many of these had previously attempted to divide or not. Probably most of them had not. At 3:45 P.M. a 7-cell stage was seen. At 9:00 A.M. of the following day most of the eggs with jelly were still in a one cell stage or in irregular early cleavage. Much of this irregular early cleavage is probably not a true cleavage but a budding. About 4 per cent. of the eggs were in a 2-cell stage of apparently normal form. A few trochophores, not at all active, were revolving feebly with a lateral displacement of perhaps twice their own diameter. They were abnormal in shape and appearance and though no cilia could be seen they were certainly present since the animals were motile. The whole culture died without developing farther. Other experiments gave similar results or produced fewer abnormalities in early development and lived to produce malformed larvæ.

Since on one day sperm which had been kept for 30 hours would cause most of the eggs in a culture to develop and upon the following day sperm only half as old might have no effect it seemed to be desirable to produce more uniform conditions for keeping the sperm. Accordingly the sperm cells were placed in an ordinary refrigerator in which the comparatively low temperature did not fluctuate so much as did that of the outer air. The best results were secured by using the sperm on the third day. By testing out the contents of a vial at intervals during the day it is possible to determine the proper time for starting an experiment. About 40 cultures obtained in this way have been under observation and have given reasonably uniform results. All of the types of abnormal development described above under the head of heat were repeatedly found in this set of experiments. The results given by this set of experiments are very convincing to one who was able to follow the material closely. It is not known definitely whether the injury to the sperm was produced by toxic bacterial products, by the accumulation of metabolic products, or by the gradual weakening of the cell by its own metabolism. Whatever the cause, I have no doubt that the effects produced were brought about by insemination with weakened sperm cells.

3. Fresh Water.—Three experiments were made in which the sperm cells were placed in dilute sea water or in distilled water for short periods of time. Sperm from mixtures containing more than 20 per cent. of sea water produced normal development. The results produced by lower percentages of sea water and by distilled water were variable, some normal larvæ appearing in cultures in which most of the eggs failed to segment. Permanent one and two cell stages were secured in large numbers. Attempts at first cleavage were also seen. Although so few experiments were made by this method that we do not get all the types of abnormality which were produced by the heat and delay, we have a clear indication that more work and better technique would produce very similar results. The work was dropped in order to take up more promising methods.

4. Alcohol.—In experiment 29 of June 29, 1911, the sperm cells were kept in a series of weak alcohols for 35 minutes. The unfertilized control showed no jelly and no segmentation. The fertilized control was normal. Sperm which had been exposed to the action of 10 per cent. alcohol (made up with sea water) were incapable of producing any effect upon the eggs. 8 per cent. alcohol gave similar results. A few eggs, about 2 per cent., were fertilized by sperm cells which had been kept in 6 per cent. alcohol. Of the 15 eggs which were observed to form jelly, 2 did not segment, one divided only once, and the remainder were motile on the second day. Of the motile forms one half were abnormal in some way upon the fourth day. During the course of the whole experiment this culture was greatly retarded in development. Sperm from the 4 per cent. alcohol fertilized 25 per cent. of the eggs and, with the exception of a few unsegmented eggs, all developed the power of motion upon the second day, though they did not develop so rapidly as did the controls. On the third day at least 3 per cent. were visibly abnormal in body form or in setæ. The fourth day showed 10 per cent. to be abnormal. Some never formed any setæ, some formed weak ones, and some were nearly perfect in this respect. Irregular bunches were often present upon the sides of the segments. The apparent age of the animals in the culture varied greatly. 2 per cent. alcohol did not prove to be so effective, for 60 per cent. of the eggs were fertilized and developed with fewer abnormalities than any of the cases given above.

Other experiments show that sperm cells exposed to weak grades of alchool for a longer time give about the same results as those exposed to higher strengths for shorter periods. Early segmentation stages do not appear to be so much affected as the larval stages, though usually there were at least a few eggs which failed to segment after jelly formation and maturation or which failed to segment more than once. There was in all cases a strong tendency for the experimental cultures to die earlier, to be less active, and to contain forms which were abnormal in body form or in setæ formation. Permanent trochophores were also common.

In this set seven experiments were completed, involving in the neighborhood of a hundred cultures. Several slightly different methods of procedure were used but the results in all cases were similar to those cited above. An extra control was carried in each experiment. This consisted of eggs and normal sperm to which was added at the time of insemination as much alcohol as was transferred to the experimental cultures along with the sperm used in insemination. In no case did this small quantity of alcohol cause this control to develop in an abnormal manner. This indicates that the abnormalities seen in the experimental cultures were not due to the presence of such minute quantities of alcohol in the egg culture.

5. Sodium Hydroxide.—In this group but few experiments were made. The sperm cells were exposed to the influence of different strengths of NaOH for a period of 20 minutes. The solutions used were made up by adding to 10 c.c. of sea water a certain number of drops of 0.75 per cent. NaOH solution. After exposure to one of these mixtures for 20 minutes the sperm cells were used for inseminating fresh eggs. Three controls were carried. The first, eggs only, showed no change. The second, eggs and normal sperm, developed in the usual manner. To the third, similar in composition to the second, was added the same amount of NaOH solution that was carried over to the eggs by the sperm in the experiment 40.4. This likewise developed in normal fashion, thus demonstrating that the trace of alkali necessarily introduced in the process of insemination of the experimental series was without any apparent effect upon early development. In table I. the results of a typical experiment are given.

The early history of the experiment was not followed on account of a lack of time. The observed abnormalities were chiefly in the failure of the trochophores to develop, in which case irregular bunches appeared on the body which became enlarged and transparent. This appearance was not due to post mortem changes, for the animals sometimes continued to swim about slowly. Many times the setæ failed to appear as has been described in other cases. In the case of 40.5 and 40.6 the smaller abnormality is probably due to the greater mortality, since the abnormal individuals are often not very resistant. The general impression given by this series is similar to that formed from the observations on the alcohol series.

TABLE I.

EXPERIMENT 40. July 21, 1911. All inseminated at 9:30 A.M.

No.	Drops NaOH.	Per Cent. Jelly at 9:55 A.M.	Time of First Cleavage.	Per Cent. Segment.	Later History.
40.1	1	100 –	10:38 A.M.	100 –	Normal.
40.2	2	100 –	10:39 A.M.	90	5 per cent. abnormal in setæ or in having permanent trochophore.
40.3	3	95	10:39 A.M.	90	About like 40.2. More weak setæ.
40.4	4	60	10:43 A.M. and scattering	40	About like 40.3.
40.5	5	40	10:41 A.M. and scattering	15	Development slower. Many die. Living less abnormal than 40.4.
40.6	6	10	11:00 A.M. and scattering	5	Like 40.5 but more abnormal and more die.

6. *Hydrochloric Acid.*—The effects of acid upon the sperm cells were also observed, the experiments being conducted in the same manner as the alkali series. A number of drops of 9 per cent. HCl were added to 10 c.c. of sea water and the sperm were placed in this solution for 25 minutes. All three of the controls were perfectly normal. Eggs inseminated by the sperm suspension containing one drop of acid often stopped development in late cleavage. Others formed irregularly shaped permanent trochophores. Later over 50 per cent. of the larvæ were abnormal in body form and character of the setæ. The eggs inseminated by the sperm suspension containing two drops of acid frequently failed to cleave. A few permanent two-cell stages were produced. Some of the abnormal larval stages again appeared but not so large a proportion as in the previous case. More than two drops

of acid affected the sperm cells so much that the eggs inseminated by the sperm cells so treated were unchanged or merely formed jelly and polar bodies without segmenting.

An attempt was made with weak NaOH solution to neutralize the acid in which the sperm cells were placed before insemination but this process so often destroyed the life of the sperm cells that it was useless for control purposes. However, the controls used seemed to be sufficient to show that the cause of the altered development does not lie in the presence of the acid in the experimental cultures of eggs.

7. Cold.—Freezing the sperm was effected by placing them in sea water in a test tube and imbedding the tube in a mixture of powdered ice and salt. − 8° C. killed the sperm. − 2.2° C. gave a mushy ice in the test tube. The sperm cells were not killed but caused a normal cleavage in the eggs. From 5 to 10 per cent. of the larvæ were abnormal. A very few scattering abnormalities of all the kinds previously mentioned were observed. − 1.6° C. did not seem to affect the sperm cells enough to alter the egg development.

Since low temperatures are very difficult to handle by means of the crude methods employed extensive experiments were not carried out along this line. Refrigeration at a temperature considerably above freezing for longer periods was also used as a possible source of injury to the sperm cells. Since the effects produced are probably due as much to the delay as to the cold these experiments have been mentioned under a previous heading.

B. Upon Arbacia.

In July, 1911, a series of 18 experiments involving about 200 cultures was carried out, using the sexual products of the common sea urchin, *Arbacia punctulata.* Along with some other investigators I had considerable trouble at that particular time in getting control experiments to develop in a normal manner. Accordingly the work was not carried as far as it seemed desirable at that time. Although the results were perfectly convincing to one who could see the living material, the small percentage of abnormality in the controls might cause some to question the value of the published data. I will, however, give a brief survey of the work since it is in line with the work upon *Nereis* and since some of the features here described have not been previously recorded.

Experiments were carried out by inseminating eggs with sperm which had been subjected to injurious conditions for a longer or shorter time. The injurious agents used were (*a*) sea water concentrated by boiling; (*b*) sea water diluted with distilled water; (*c*) sea water with from one to twelve per cent. of alcohol added; (*d*) sea water slightly acidified with HCl; (*e*) keeping the sperm cells from 6 to 24 hours after removal from the body; and (*f*) combinations of the above.

In a general way these methods of treatment did not give specific effects but produced very similar results, although the intensity of the effects varied as was to be expected. In one case the controls were nearly as abnormal as the experimental cultures. But, taking the experiments as a whole, there can be no doubt as to the great differences to be found between the controls and the experimental series.

The abnormalities produced by inseminating normal eggs with injured sperm may be briefly reviewed. In a few cases in which the sperm cells were exposed to extreme conditions, insemination gave a number of cases of membrane formation without development. Cleavage was found to be extremely irregular in some cases, producing fantastic forms, such as ciliated plates and rows of cells. The more normal cleavage was also found to be irregular but it is difficult to say that it was more irregular than it sometimes is in normal cultures. The development of the experimental series was often found to be retarded, especially after late cleavage. The early cleavage was not affected so much, though occasional indications of a slight retardation were observed. Curiously enough, in some of the experiments using alcohol, the experimental material was observed to be one or two cleavages ahead of the controls in the early segmentation stages, though the difference was soon in the other direction, if any was apparent. Prismatic forms were often observed in the controls when the experimental cultures were chiefly in the blastula stage. The death rate was much higher in all of the experimental series, the whole culture sometimes dying in various stages from blastula to pluteus before any considerable number of deaths were observed in the controls. A very small number died during cleavage in stages beginning with the undivided egg. Blastulæ whose cavities were more or less filled with cells, similar to the stereoblastulæ so often described by experimental workers, were found in increasingly large percentages as the strength or duration of the injurious agent acting upon the sperm was increased. In some cases over 15 per cent. of the experimental cultures were of this form. They did not appear to develop farther but went to pieces. Evaginate gastrulæ were formed rather uniformly throughout the experimental series and occasionally one or two were found in the controls. The proportion of this type of abnormality did not seem to show so close a correlation with the degree of injury to the sperm as did the stereoblastulæ. Other types of abnormal gastrulæ were very common, this stage seeming to be a difficult one to pass. Some were observed to be much smaller in size than the normal type, perhaps due to a separation of the blastomeres in the early stages. Others were irregular in various ways. The most common type of this irregularity is what I have called in my notes "ragged." These forms do not possess such a clean cut appearance but are covered with minute irregularities and look as though they were going bad. The plutei were variable in the controls, but they were very much more so in the experimental cultures. The arms sometimes failed to form or appeared only as small buds. Usually however the arms were longer and more slender than in the controls. Several cases were observed in which the anal opening did not form. In all cases after the first appearance of motility a much larger number was found in the experimental cultures lying motionless on the bottom of the dish. If disturbed they quickly settled down to their former condition of rest. Some seemed to entirely lack the power of motion although this was not certainly determined.

In general all of the results point toward a great disturbance in the development of all or nearly all parts of the organism. This disturbance seems to be produced by injury to the sperm cells used in insemination. An attempt to see if, in the case of the experiments using alcohol, the disturbance was due to a possible trace of alcohol carried over with the sperm in the process of insemination, was made by adding alcohol to the control cultures at the time of insemination. The addition in quantities comparable to the amount present in the experimental cultures was without effect. Table II. gives the principal features of a representative experiment, the minor features being omitted.

TABLE II.

EXPERIMENT *A*2. July 4, 1911. Sperm removed at 9:00 A.M. from a male with testes about half soft. Two drops of thick sperm placed in 10 c.c. of each grade of alcohol at 9:15 A.M. and left there 5 hours and 20 minutes. Eggs about 2 per cent. immature. Used immediately after removal. All except *A*2.0 (the uninseminated control) inseminated at 2:35 P.M. Discontinued July 7, 1911.

No.	Per Cent. Alcohol.	Condition at 3:30 P.M., July 4.	Condition at 11:00 A.M., July 5.	Condition at 2:20 P.M., July 5.	Condition A.M., July 7.
*A*2.0	0	Normal.	Normal. No cleavage.	No cleavage. Breaking down.	
*A*2.1	0	2-cell.	Normal gastrulæ.	Late gastrulæ. Trace abnormal.	10 per cent. abnormal.
*A*2.2	2	2-cell.	Gastrulæ 10 per cent. ragged.	Many abnormal late gastrulæ. Ciliated plates seen.	Mostly abnormal.
*A*2.3	3	2-cell.	Gastrulæ 50 per cent. ragged. 1 per cent. stereoblastulæ. A few 2 cells. 1 morula.	As at 11:00 A.M. only more developed.	60 per cent. very abnormal.
*A*2.4	4	2-cell.	40 per cent. abnormal. 1 per cent. stereoblastulæ.	As at 11:00 A.M. but more developed.	80 per cent. abnormal.
*A*2.5	5	2-cell.	Mostly abnormal gastrulæ. 4 per cent. stereoblastulæ.	As at 11:00 A.M. but more developed.	All dead.
*A*2.6	6	No cleavage.	Trace swim. 5 per cent. stereoblastulæ.	As at 11:00 A.M. but more developed.	All dead.
*A*2.7	7	No cleav. age.	None swim. 11 per cent. stereoblastulæ.	Dead.	
*A*2.8	8	None fertile.	None fertile.		

V. CYTOLOGICAL STUDY.

A. Normal.

I have found it necessary to go into some of the cytological details which are to be found in the egg of *Nereis* after insemination with normal sperm. These features have been described previously (Lillie, F. R., '11, '12) but an outline of the cytological changes is necessary for a proper understanding of the observations on the experimental material. My studies have been based upon my control series and upon slides loaned to me by Professor Lillie. The latter are especially fine and show clearly all of the conditions which he has described in his papers. Immediately after a spermatozoön becomes attached to the egg by means of its delicate perforatorium the contents of the coarse alveoli of the cortical layer of the egg begin to pass to the exterior where, in the course of fifteen minutes, they form a thick layer of jelly. A perivitelline space now occupies the position formerly held by the cortical layer of the egg. An entrance cone forms from the egg cytoplasm just beneath the spermatozoön and, about three quarters of an hour after insemination, draws the sperm head, in the form of a thread, into the egg, leaving the tail and middle piece outside. This usually occurs during the late anaphase of the first maturation spindle. The sperm head now begins to grow larger and, retaining its connection with the entrance cone, penetrates the egg protoplasm. The whole complex, as it passes to the yolk free area of the egg, rotates so as to bring the sperm nucleus ahead and nearer to the center of the egg. Meanwhile the sperm aster appears at the pole of the nucleus which is opposite the cone and soon divides, forming an amphiaster. Following the formation of the second polar body, the egg aster fades away and the egg chromosomes swell and form conspicuous vesicles, each one with a distinct chromatic nucleolus, which slowly fuse together to form the egg nucleus. By this time the sperm nucleus has increased in size and presents an appearance much like that of the egg nucleus, near which it has come to lie. The two nuclei now fuse and the asters of the first cleavage spindle, presumably derived from the sperm amphiaster, are seen on opposite sides of the cleavage nucleus. The process of cleavage then ensues.

B. Experimental.

In the experimental material it has proved difficult to establish a definite seriation in the cytological observations, since, as the observations on the living material indicate, in the experimental cultures different eggs start to develop at different times. This seems, at least in part, to be due to the relatively low activity of the injured sperm cells and to the consequent delay in attachment. Likewise it has been hard to get any satisfactory estimation of percentages of the various stages present in any preservation. The reason for this lies in the fact that in most cases where marked results were obtained, only a small fraction of the eggs were fertilized and too few fertilized eggs were secured from any one series to get counts large enough to be significant in such complex conditions. The losses in sectioning and staining also add to the difficulties. Yet it has been possible to secure the main facts by going over a large number of slides and by comparing the results of different experiments.

Cytological study of the preserved samples from the experimental cultures of *Nereis* eggs shows the following classes of eggs.

(*a*) Eggs not entered by sperm. No segmentation.
   1. Polar bodies formed. Jelly not extruded.
   2. Polar bodies formed. Jelly extruded.
(*b*) Eggs entered by sperm. Segmentation.
   1. First cleavage not completed.
   2. Multipolar mitosis. Maturation incomplete.
   3. Multipolar mitosis. Maturation completed. Polyspermy.
   4. Miscellaneous abnormal eggs.
   5. Apparently normal eggs many of which would doubtless have proved to be defective had they been allowed to develop.

Cytological study reveals nothing as to the cause of the abnormalities occurring after the time of the first cleavage, with the single exception of the above mentioned multipolar mitosis.

(*a* 1) As is indicated by the records made from examination of the living material, by the India ink method, a partial or total lack of jelly formation is one of the striking things which is to be found in nearly all of the experiments. I have never seen this in normally fertilized eggs, either whole or in sections, although egg sections from early preservations of the experimental series show in some slides as many as 5 per cent. of the maturing eggs with the alveoli of the cortical layer still wholly or partially filled. In most cases an attached sperm cell may be found outside the vitelline membrane. Since it is in an exposed position, not protected by jelly as in other eggs, its absence may be interpreted as due to its accidental removal after preservation. I have not found fertilization cones in sections which show any considerable amount of material still present in the cortical alveoli. Maturation goes on in an apparently normal fashion (Fig. 1) though probably much slower than usual since the later maturation stages of this type are not seen until the normal maturation stages have been completed for some time. I have found most of the principal maturation stages, from the breaking down of the germinal vesicle to the formation of the vesicles of the female nucleus, and, with the exception of the cortical layer, they seem to be like the normal stages. The polar bodies either come to lie imbedded in the cortical layer, or are pushed entirely through it to the outside (Fig. 2). After the formation of the second polar body the chromosomes, in the usual manner, rapidly form a number of large chromosomal vesicles with the haploid number of chromatic nucleoli. But no indication of the sperm is to be seen within the egg. No trace of cytoplasmic radiations is ever seen within the egg after the maturation phenomena are completed and the sperm nucleus is not to be found unless the remnants of the sperm cell are still attached to the outside of the vitelline membrane. The chromosomal vesicles, not being able to fuse with the sperm nucleus, may now behave in one of several ways. They may scatter at random through the cytoplasm or they may retain their position beneath the polar bodies, in which case they usually form a more or less hazy, poorly staining mass of irregular shape. In a few cases I have found about a dozen separate or partially fused vesicles containing in all about 26 chromatic nucleoli. At first I thought that this was a case in which the sperm had entered. But the entire absence of cytoplasmic radiations, the irregular arrangement of the vesicles, and the determination of approximately the haploid number of vesicles indicates that we are dealing with a case of multiplication of the chromatic nucleoli without true fertilization. In some cases the chromosomal vesicles seem to dissolve in the cytoplasm, leaving behind several small chromatic nucleoli.

To sum up, the sperm attaches itself to the egg which slowly undergoes maturation but does not form jelly or segment. The sperm does not enter the egg and no fertilization cone is formed. After the formation of the second polar body, the chromosomal vesicles of the egg nucleus form and, failing to unite with a sperm nucleus, undergo degenerative changes. The spermatozoön thus gives the egg a stimulus which induces only the formation of the polar bodies. In such cases the maturation process goes on in a normal manner with the exception of a retardation in the rate. This retardation is probably caused by the retention of the jelly-forming materials in the cortical layer of the egg, which would tend to cut down the rate of exchange with the external medium.

It is possible that we are dealing in this case with eggs which require a rather high degree of stimulation in order to produce jelly. Observation on unfertilized eggs shows that there is a considerable degree of variation in the amount of stimulus required to produce jelly formation. It may be that the injured sperm cells are incapable of giving so great a stimulus for jelly formation as do the normal ones and that some of the eggs cannot respond to the slight stimulus given, by emptying their cortical alveoli, although they may be able to form polar bodies. Whether the cause lies in the egg or in the sperm it is necessary, since these eggs develop normally if fertilized with uninjured sperm, to assume that the injury to the developmental process is produced by the action of external influences upon the sperm cell before the time of insemination. It is also clear that in the case of *Nereis* eggs maturation may take place without jelly formation.

(*a* 2) In a much larger number of cases the eggs go a step farther than those described above. The living material shows eggs which undergo both maturation and jelly formation but which never segment. Although, in the earlier stages, a sperm cell may be seen to be attached to the vitelline membrane no fertilization cone is present. Examination of the sections of such eggs completely confirms the observations made upon the living material. The sperm cell becomes attached to the vitelline membrane, the processes of maturation and jelly formation follow in a normal manner, and the animal pole of the egg becomes free from yolk. But no fertilization cone is formed and no attachment granules appear at the end of the perforatorium (Fig. 4). The egg protoplasm just beneath the attached sperm shows no more change than that at any other part of the egg. The perforatorium, which in cases of normal attachment becomes wider and more easily seen (Fig. 5), remains as a very delicate process which cannot be followed beyond the vitelline membrane. Large granules, which stain deeply in the iron hæmatoxylin, soon appear in large numbers in the peripheral parts of the egg and also around the egg nucleus. In later stages the sperm head becomes larger and somewhat less dense and often disappears entirely. This is presumably due to secretions which are thrown off from the egg, as has been demonstrated by F. R. Lillie ('12).

No evidence of the presence of a sperm within the egg can be found. At the close of the second maturation period the egg chromosomal vesicles begin to swell and may unite with each other to a greater or less extent (Fig. 3). Cytoplasmic parts of the mitotic figure never appear although there are sometimes traces of chromosome formation within the vesicles in the earlier stages (Fig. 6). In some cases the vesicles separate and scatter through the cytoplasm where they may dissolve and leave behind a number of deeply staining chromatic nucleoli. More often the vesicles remain in place and break down into a mass of poorly staining debris (Fig. 7) in which, in the earlier stages, chromosome-like forms may be visible. Two or three hours after insemination the cytoplasm frequently begins to bud off small pieces filled with deeply staining granules. This usually appears first near the equator. Later the whole cell may break up into parts which more or less resemble blastomeres, although close examination in the living state reveals their nature. In sections it is easily seen that the parts do not possess nuclei.

It is clear that the injured sperm has given the first stimulus of fertilization. But, although the initial stages have been produced, the effects necessary for further development have not been called forth within the egg. The achromatic parts of the mitotic figure are entirely absent although there are indications that the chromosomes may undergo a part of the changes preliminary to first cleavage The effects produced by the attachment of the sperm seem to be nearly the same as those secured by centrifuging the eggs or by giving them any mechanical stimulus (Lillie, '11).

It is of interest to find that a few of the eggs of the type described above show the presence of two or more sperm cells attached to the vitelline membrane. This has been found in several cultures in which many eggs showed no evidence of having come in contact with a sperm cell. Apparently the weakened sperm cells are not able to promptly call forth in the egg the reaction which prevents the attachment of more than one sperm. The partial jelly formation which was observed in the living material is often associated with the failure of the sperm to enter the egg. However cases have been seen in which the sperm entered and caused first cleavage to take place. The farther history is not known but, since preservations made from somewhat later stages show no traces of such eggs, it is probable that the cortical alveoli are soon, emptied.

(*b* 1) A very interesting observation upon the living material has been recorded above. In many experiments a few eggs seemed to have nearly completed the process of first cleavage when the cleavage plane ceased to advance and gradually faded away. Cytological examination fails to pick out these eggs until the early telophase stages of first cleavage. The earlier processes seem to go on in a normal manner. In most cases the chromosomal vesicles fail to unite properly and the plane of cleavage fades away. Usually it leaves behind a change in the arrangement of the yolk granules and its position is occupied by a somewhat lighter staining band in which small granules are less abundant. Parts of the sharp line which marks the completed cleavage plane in sections may be left behind, especially those parts near the animal pole of the egg (Fig. 8). The chromosomal vesicles sometimes scatter widely and partially dissolve and sometimes they remain near each other. Very often the region near the vesicles becomes filled with deeply stainirg granules. In one case a cell was found in the late prophase of second cleavage, though without a first cleavage plane. In this case the nuclei have been able to divide again though the cytoplasmic division was never completed. In most cases the cells which fail to finish the first cleavage never go any farther.

(*b* 2) A very few eggs were found in each of the experiments which exhibited the above described attempted cleavage, in which multipolar spindles were found. Unfortunately my preserved material contains very few of these eggs and the sections of some of them were lost or badly broken in the process of preparation.

There are two different kinds of eggs which show this condition. One kind has formed two polar bodies. There are very few of these eggs which are in a condition good enough to work upon. Although the cultures from which the eggs were taken were more than 75 per cent. unfertilized these eggs seem to show evidences of polyspermy. This is borne out by the fact that, in a few cases, two sperm cells were seen to be attached to a single living egg in cultures similar to those from which these eggs were taken. Although it is conceivable that multipolar figures might arise from causes other than polyspermy, it is very unlikely. I regard the few observations of polyspermy in the living cultures as good evidence that the few cases under consideration are due to the entrance of more than one sperm cell. The observations mentioned under (*a* 2) above also point in this direction.

(*b* 3) The other kind of multipolar figures is found in eggs which show defective maturation. In one case an egg was found in which the first polar body had formed. Just beneath it lay 14 chromosomal vesicles and in the center of the section about 20 vesicles lay near a strong cytoplasmic radiation. The presence of the radiation probably indicates that a sperm nucleus has fused with the egg nucleus, although the number of vesicles which can be made out is below the diploid. Another sperm cell lies in contact with the vitelline membrane, probably attached to it. Although this is not a case of multipolar mitosis in itself it is possible that the 14 vesicles near the animal pole, which represent the second polar body, may enter into the cleavage process and aid in the production of multipolar figures. One or two cases of such figures in eggs with but one polar body have been noted in late cleavage stages. Other eggs may fail entirely to produce polar bodies. In these eggs from six to nine poles form and the spindles are often very complicated and produce an irregular distribution of the chromosomes, which, in the earlier figures, take the form which is found on the second maturation spindles. It has been shown (Lillie, F. R., '11) that the formation of the first polar body may be prevented by the use of the centrifuge. In such cases multipolar figures of various kinds are formed. Clearly my case is due to the suppression of the polar bodies. I have not seen similar cases in sections of normal series though, in such small numbers as are found in the experimental series, it is possible that they may have been overlooked.

(*b* 4) A few scattering abnormalities have also been found but not in sufficient numbers to be of any significance. Two cases in which the sperm and egg nuclei fused together and failed to develop farther have been seen. Two cases of prophase figures from second cleavage were found in which the spindles lay at right angles to each other in different planes. Other abnormal conditions were also found. All of these cases are open to the interpretation that the egg was abnormal before fertilization and are not worth description.

Careful search was made for eggs showing abnormalities at the period of entrance of the sperm. It seemed likely that the stages of fusion of the egg and sperm nuclei would contain evidence of abnormality but close examination failed to show anything of the kind, except in the case of the two eggs already mentioned. Since a kind of parthenogenetic cleavage might be produced by the partial action of the sperm cell, search was made for eggs exhibiting the haploid number of chromosomes in the first cleavage. But in all cases the number was clearly diploid.

VI. Discussion.

A. The Production of Defectives.—For a long time it has been assumed by many that the offspring of parents who habitually take alcohol or other drugs or who work in lead are very apt to be in some way weak or defective. Some of the unfortunate conditions in the offspring which different men have associated with parental alcoholism are insanity, epilepsy, feeble-mindedness, cretinism, macrocephaly, lack of self-control resulting in criminality or in over-indulgence in alcoholic drinks, malformations, retarded development, poor health, and the like. Medical men often state that there is every indication that a great number of abortions, stillbirths, and births of defectives are definitely connected with times of conception corresponding to periods of drunkenness, or drug craze, or of working in lead. So much has been written on both sides of the question, especially with reference to alcohol, that it is impracticable to attempt to give any exhaustive review of the literature. Unfortunately, also, so much of the writing is the product of prejudice and of a desire to establish some point, regardless of the evidence, that it is difficult to separate the wheat from the chaff.

There are three theories to be mentioned in connection with the relation of alcoholism to the production of defectives. (1) Since the excessive use of alcohol unquestionably produces undesirable effects upon the body of the consumer, many believe that the offspring of an alcoholic parent may in some way inherit certain weaknesses thus produced. But it seems that at present we have no reason to believe that *specific* somatic alterations of the parent, produced by drugs or otherwise, are inherited by the offspring. Consequently any such theory may be rejected at once.

(2) Some writers go so far as to assert that there is no direct *causal* connection between parental alcoholism and defectiveness in the offspring. They maintain that alcoholism, insanity, feeblemindedness, epilepsy, etc., are often merely different forms of expression of a single weakness. In many cases, as stated by W. Branthwaite, H. M. Inspector under the Inebriates' Act ('08), alcoholism is undoubtedly caused by some weakness which is present in the family. There seems to be sufficient evidence to show that this statement is true. Yet we cannot accept the theory that this hereditary weakness entirely explains the relations under discussion.

(3) Very few writers have even ventured to suggest that the defective conditions appearing in the offspring of alcoholics

may be due in part to the direct action of the alcohol upon the germ-cells; in case the father alone be alcoholic that his spermatozoa may be so affected as to induce abnormal development of the ovum fertilized by him. Until recently there has been little evidence to show that such a theory is tenable and more evidence is desirable. The data presented in this paper demonstrate that the sperm cells of *Nereis* and *Arbacia* may be so affected by alcohol and by other methods of treatment, that their union with normal eggs will produce an abnormal development. As is shown below, there is evidence of a similar nature in other forms.

In man the conditions are so complex that they are hard to analyze, especially since we may not resort to direct experiment. Alcohol poisoning may take place in utero, during the nursing period, or even later. Or it may be that the same depressing conditions which caused the parents to drink may react similarly upon the offspring. Many other difficulties also confront the investigator who seeks to solve the problem by the methods of the past. It seems clear that such methods will never give the solution. Definite biological experiment upon the lower animals must form the chief basis for any conclusions which we may make in the future. With this thought in mind the present series of experiments was undertaken in the hope that some definite evidence might be presented upon the subject.

Although there may sometimes be a suspicion that the data relating to man have been selected with a view of establishing a point rather than seeking the truth, it is worth while to recall a few cases because of their interest in the present connection. Several European investigators (*e. g.*, Schweighofer and Bezzola) are frequently quoted in the literature as having found that there is a definite relation between the time of the greatest number of stillbirths, abortions, and births of mental defectives, and the great feast seasons, during which much alcohol is consumed. From a statistical standpoint such statements have been severely criticized, especially by Pearson and Elderton ('10). There is also little evidence to show that the effect of the alcohol was not exerted upon the developing embryo. Since the effect of the alcohol taken by the mother may be either upon the egg or upon the developing organism, the most desirable evidence comes from cases in which the male germ cells alone are exposed to the supposedly unfavorable conditions.

Saleeby ('11) quotes Galton as having given the three following cases. A man who had normal children became a drunkard and his later children were all imbeciles. A healthy woman who had by a drunken husband five sickly children who died in infancy, later married a healthy man and produced normal children. A man with two healthy children acquired the cocaine habit and engendered two idiots.

Schweighofer gives a case of a normal woman who had three normal children by a sound man. She later married a drunkard. Of the three children from this union one had infantilism, one was a drunkard, and one was a degenerate. In a third marriage she again bore healthy children.

Paul ('60) tells of the children of lead workers. From 32 pregnancies, the father alone being exposed to the lead poisoning, there resulted 12 abortions, stillbirths, and premature labors, and 20 living births. Of the living 8 died during the first year, 4 during the second, and 5 during the third. Paul states that the influence of the lead is as real as in the cases where the mother is exposed, though perhaps the effects produced are not so great.

The observations quoted above are among the best on record. Many other similar observations can be collected by anyone who thinks it worth the time. A rather full bibliography is given by Hoppe ('12). In the light of recent work these facts are very interesting. Although in some cases the remarriage serves as a control, the lack of data concerning the previous family histories is a defect sufficiently serious to warrant us in questioning any conclusions which may be drawn from these data alone. So far as I have searched there are no observations upon man which meet with rigorous scientific requirements.

Elderton and Pearson ('10) conclude from the statistical study of English school children that the offspring of alcoholic parents are slightly brighter, heavier, and less diseased than those of sober parents and that epilepsy and tuberculosis are of no more frequent occurrence than among the children of non-alcoholics.

They also record a higher mortality among the children of alcoholics and conclude that the more resistant survive. The publication of this paper has been followed by widespread criticism. The details of the individual cases are not given. We do not know what may be included under the terms "alcoholism" and "intemperate." We should know the condition of the children who were not in school, due perhaps to lack of ability or to ill health, the relation of the time of conception to periods of drunkenness, and whether the mother, the father, or both, were alcoholics. In view of the lack of so much desirable evidence and of the heterogeneity of the materials investigated it seems that the question as to the general applicability of the conclusions reached is at least an open one, the more so since the experimental evidence seems to be directly opposed in most cases. Additional light is given by the researches of Nicloux ('00) who proves that alcohol may reach the ovaries and testes of mammals and that these organs take up considerable quantities of the drug. Alcohol is also present in the seminal fluid very shortly after it is taken into the stomach. Accordingly it seems to be possible that the sperm cells may be injured. Bertholet's work ('09) in a sense confirms that of Nicloux, since he finds that testicular atrophy is common among alcoholics. His observations lead one to think that sterility should be much more common than it is among drunkards.

There are a number of published observations upon the lower animals. Mairet and Combemal ('88) foumd that a dog treated with absinthe for 8 months and paired with a normal female gave 12 young. 2 were born dead and the others all died within 11 weeks after birth. The small numbers and the lack of adequate control make this experiment indecisive.

Bardeen ('07) found that toad eggs, when fertilized by sperm cells which had been previously exposed to the X rays, developed abnormally. Since the question involved is wider than the alcohol question these results are significant.

O. Hertwig ('10, '11) and G. Hertwig ('12) obtained similar results by the exposure of the sperm cells of various animals to radium and also by injecting methylene blue into the dorsal lymph sac of the male frog some days before the sperm cells were used to fertilize normal eggs. In this work it was found that the stronger action upon the germ cells produced earlier and more profound alterations in the offspring.

Stockard ('12) furnishes a set of well-planned experiments upon guinea pigs which, when tested, produced normal offspring. The males were intoxicated by inhaling alcoholic fumes. As a result of 24 matings of alcoholic males with normal females he reports no results or early abortions in 14 cases, 5 stillborn litters, and 5 living litters containing 12 young. Of these, 7 died shortly after birth. The remaining 5 "are unusually small and very shy and excitable animals." The parents remained in good health throughout the experiment. Although the number of matings is rather small there is a clear indication that the action of the alcohol upon the male germ cells is the cause of abnormal results. Considered in connection with the work of Bertholet and of Nicloux this is very strong evidence.

The experiments of Nice ('12) do not seem to entirely agree with the experiments just quoted. His mice, both male and female, were fed on milk and crackers, to which 2 c.c. of 35 per cent. alcohol was added daily. They were also furnished with drink in the form of 35 per cent. alcohol. The fecundity of the mice was greater than that of the control series, though the mortality was 11.1 per cent. in the offspring in the experimental series and zero in the controls. None of the young were deformed. Nicotin, caffein, and tobacco fumes gave similar results. In the absence of farther experimental data it seems fair to assume that the germ cells of the mice are in some way less susceptible to these drugs, though the comparatively high mortality indicates that there was an effect.

Gager ('08) subjected pollen grains to the action of radium and secured a marked change in the plant resulting from pollination of a normal plant. Some of the effects persisted through several generations.

My own experiments upon *Nereis* and *Arbacia* demonstrate that injury of the sperm cells by several methods may produce a series of abnormalities which may appear in various stages from the time of insemination up to late larval stages. It is not known whether, if the animals were kept under favorable conditions for life and growth, other abnormalities would occur in later stages, but since experimental cultures which are apparently normal in the earlier stages frequently develop abnormalities in the larval condition, it is very probable that other changes would come to the surface in still later stages. In the *Nereis* experiments there can be no doubt as to the cause of the abnormalities observed. The controls, using the sexual products from the same animals, exclude the possibility of abnormalities being present in the lines used. The very large numbers handled demonstrate that the results are not due to the chance outcropping of hidden defects. The series composed of *Arbacia* material, if standing by itself, might be questioned. But the general type of results is so similar to that obtained by Bardeen, the Hertwigs, Stockard, and myself on other forms that there can be no question as to their applicability.

The lack of specificity in the action of the agents used is remarkable. At first thought it seems strange that acid, alkali, alcohol, heat, delay, and other means should produce similar results. It is clear that the action must be much the same in all the cases which I have recorded as well as in those recorded by the authors cited. At present we can neither assign a definite reason for the lack of specificity in the results nor tell how the abnormalities are produced. I can only suggest that the sperm cells are affected in such a manner that their vitality is lessened, or in other words, their rate of metabolism is lowered. It is known that the rate of metabolism in the normally fertilized egg rises rapidly after the time of fertilization and continues to rise for some time. Child ('11) has shown that in the regulation of pieces of *Planaria* the type of structure formed may be definitely controlled through changes in the rate of metabolism produced by means of low temperature, anæsthetics, carbon dioxide, etc. He finds that, in a general way, regions of normally low rate, such as posterior regions, are most affected. Any particular process of morphogenesis seems to require a certain minimal rate of metabolism for its normal completion. It is possible that we are dealing with a similar case. The injured sperm cell may be unable in some cases to give to the egg a sufficient stimulus to raise the rate of metabolism to a point where it is able to draw in the sperm head. In other cases the sperm head enters and the rate may be increased greatly but not enough to cause the normal completion of many processes. In some cases the rate may be so low that cleavage cannot take place in a normal manner. Since nearly all of the forms investigated show abnormalities at the period of gastrulation it may be that the minimal rate necessary for normal gastrulation is often not reached by the eggs in the experimental cultures. In *Nereis* at the period of elongation following the trochophore stage, there is again a production of abnormalities in the posterior region. Although the evidence is very fragmentary at present it seems that the lack of specificity in these experiments is perhaps capable of explanation upon the basis of lowered rate of metabolism produced by injury to the sperm. Farther research will be necessary before we can do much more than venture a guess as to the solution of the problem.

We are safe in concluding from the observations upon man and the higher mammals and from the experimental work upon mammals, amphibians, annelids, echinoderms, and the higher plants, that it is possible to injure the male germ cells by the application of external forces so as to produce a change in the next generation at least. We cannot say just how long this change may persist. The work of Gager upon plants indicates that changes so produced may persist through several generations. Tower's work upon the female germ cells of *Leptinotarsa* indicates that the changes may persist or may gradually fade away. In all probability the results obtained by the methods which I have used will prove to be for the most part transitory, although there is no reason to believe that some of them may not persist.

There is, then, good reason to believe that some drugs, such as alcohol and cocaine, are a detriment, not only to the consumer but also under certain conditions to his offspring. Since alcohol appears in the seminal fluid very shortly after being taken into the stomach, there is good reason to believe that a man, intoxicated for the first time, even, may beget offspring which will be in some degree defective. It is also possible, though not demonstrated, that, in addition to drugs taken voluntarily into the system, the products of abnormal metabolism may exercise a similar influence. It is even conceivable that nervous states may be able through alteration of somatic metabolism, to affect the germ cells. Many other possible causes of abnormality might be mentioned. But speculation is useless at this time and experimentation is needed. A large field for investigation is opened up and the results of experiments in this field cannot fail to be of interest to the student of eugenics. The sociological application of the observations here recorded is sufficiently obvious.

B. Fertilization.—The results of these experiments are completely in accord with those given by F. R. Lillie ('11 and '12). So far as my observations extend they are practically identical with those given by Lillie. He finds that if the attached sperm cell is removed by centrifuging, the processes of jelly formation and maturation go on in a normal manner, though cleavage does not result. My experiments demonstrate that if the sperm is injured so much that it fails to enter the egg, essentially the same results are secured. This fact again supports the view expressed by Loeb ('09), Lillie ('11 and '12) and Bataillon ('12) to the effect that at least two factors are involved in the process of fertilization. The first is concerned with membrane formation and, in itself alone, is insufficient. Certain cytoplasmic changes such as the rearrangement of the yolk granules are also produced by the initial stimulus. Since slight stimuli cause jelly formation and maturation, pricking as in Bataillon's experiments is probably sufficient to produce these changes. Certainly it is difficult in *Nereis* to see any action of the sperm beyond the attachment of the perforatorium, which is responsible for the early changes in the egg. In *Nereis* it is evident that maturation may take place in the absence of membrane formation if the stimulus given by the sperm is sufficiently light. The second factor has to do with the internal stimulus. It is a difficult matter to determine at just what stage fertilization is complete. In all cases which I have observed, the formation of a fertilization cone is succeeded by the entrance of the sperm head and by the formation of the first cleavage spindle. The internal stimulus is not yet completed, even in the case of fertilization by a normal sperm cell at the time when the fertilization cone is formed. This is shown in

Lillie's experiments by the removal of the sperm cell at this stage. Since the formation of attachment granules and a fertilization cone is not in itself necessary for membrane formation, maturation and other visible cytoplasmic changes, as is shown by my experiments, and since their presence without the sperm head, does not lead to a greater visible change than does their absence, there is good reason to believe that they function solely in the attachment, penetration, and revolution of the sperm head. After these functions have been performed they cannot be traced much farther. They are probably dedifferentiated and behave as ordinary cytoplasm. I am inclined to think that in *Nereis* the mere penetration of the egg cytoplasm by the sperm head is insufficient although the observations on this point are not entirely satisfactory. The experiments of Ziegler ('98) and Wilson ('03) support this view. Ziegler succeeded in producing a constriction in the egg of the sea urchin in such a way that it separated the egg nucleus from the sperm nucleus. The part containing the sperm nucleus segmented. The remainder failed to segment, but gave indication that the presence of the sperm nucleus was not without effect, by dissolving and reappearing several times.

Wilson cut the eggs of *Cerebratulus* in two, shortly after the penetration of the sperm cell. When the cut separated the two nuclei the part containing the sperm nucleus segmented and the other part formed polar bodies but refused to cleave.

Even in cases where the germ nuclei of *Nereis* copulate there is not necessarily a complete stimulus to development. F. R. Lillie thinks that the partial sperm nuclei produced by centrifuging at the proper time do not cause normal cleavage. Sometimes a partial cleavage results and sometimes cleavage ceases in the two-cell stage. In my experiments many eggs either attempted to cleave and failed or stopped after cleaving once. This indicates that the mere presence of the germ nuclei is insufficient as an internal stimulus for development. The normal interchange between the nucleus and cytoplasm is not necessarily brought about. Although the nucleus of the sperm succeeds in producing aster formation yet the rate of metabolism is so low that normal cleavage cannot take place or can take place only once or twice. Although we have no direct evidence as to the comparative rates of metabolism of eggs fertilized with normal sperm and those fertilized with injured sperm it seems possible that a difference exists. The retardation in development, the higher mortality in the later stages, and the differences in phototropic response indicate that this is likely.

VII. Summary.

1. *Nereis* eggs, inseminated with sperm cells which have been injured by one of several methods, may fail to develop in a normal manner.

2. In some cases the egg does not form a fertilization cone, attachment granules are lacking and the sperm head is not drawn into the egg but remains outside attached to the vitelline membrane. There are two classes of such eggs, neither of which segment, viz.: (*a*) Those which slowly undergo maturation without forming jelly. (*b*) Those which form both jelly and polar bodies.

3. Those eggs in which the sperm head enters, form the first cleavage spindle in an apparently normal fashion but may fail to complete the division of the cytoplasm. Those which complete this division may develop abnormalities in later stages.

4. The eggs of *Arbacia* exhibit a similar series of abnormalities when fertilized by weakened sperm cells.

5. There is no indication of specificity in the action of the agents used in injuring the sperm cells.

6. These experiments demonstrate that eggs fertilized with sperm cells injured by alcohol and by other means may produce abnormal forms. Taken in connection with the demonstration by others that the germ cells of mammals may be exposed to injurious conditions this has an important bearing upon the relation of alcoholism to the production of defectives.

7. There seem to be at least two factors involved in the process of fertilization. The one has to do with membrane formation and certain other changes, the other with the internal stimulus.

8. In *Nereis* the presence of the two germ nuclei within the egg is not necessarily sufficient as an internal stimulus for normal development.

I am indebted to Prof. Frank R. Lillie for suggesting this line of work and for his many kindnesses during the progress of the work.

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EXPLANATION OF PLATES.

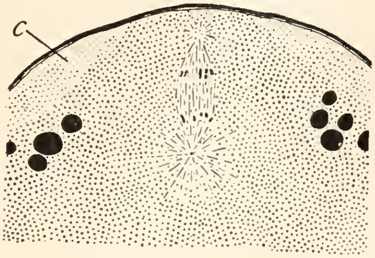
PLATE I.

All figures were drawn with the camera lucida with Leitz apochromat 2 mm. oil immersion objective, and Zeiss No. 6 compensating ocular. *c*, cortical layer.

FIG. 1. Egg fixed 1 hour and 20 minutes after insemination with sperm which was injured by heating to 43–44° C. for 8 minutes. No jelly has formed. Peripheral alveoli still intact. Anaphase of first maturation.

FIG. 2. Reconstruction of 2 sections of an egg from same culture as Fig. 1, fixed 1 hour and 50 minutes after insemination. Both polar bodies have been formed. Peripheral alveoli still unemptied. Egg nucleus in form of scattered vesicles, only part of which are seen in this figure. In all, 14 vesicles and about 28 nucleoli are indicated. Sperm cell (in another section) is still attached to vitelline membrane.

FIG. 3. Egg fixed 1 hour and 15 minutes after insemination with sperm which was injured by heating to 43–44° C. for 17 minutes. Jelly was formed and both polar bodies are present. Second polar body shown. Egg nucleus in form of vesicles, 13 of which are indicated in the various sections. Sperm cell (in another section) is still external. Strongly staining granules around periphery of egg.



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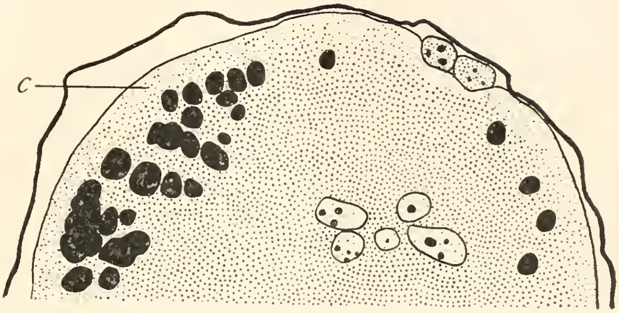

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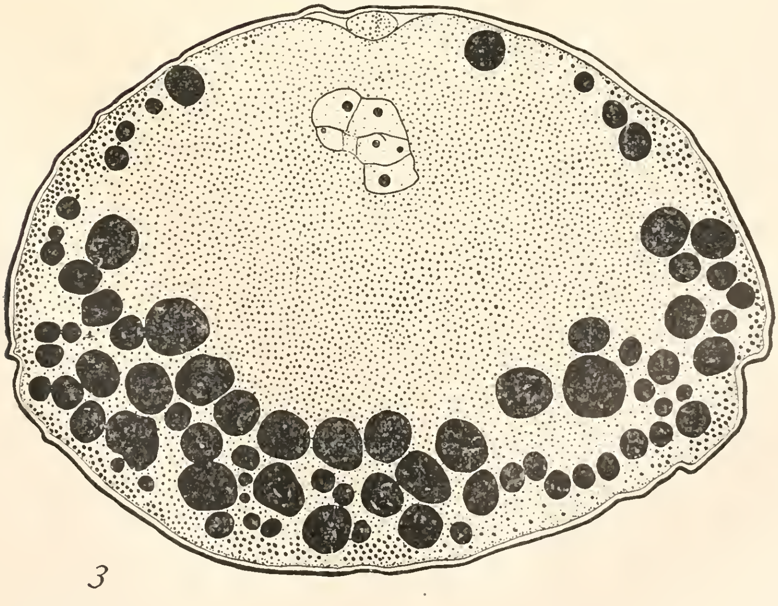

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NEIL S. DUNGAY.

PLATE II.

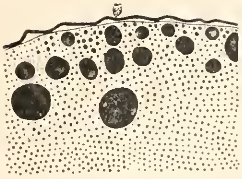
FIG. 4. Egg, from same culture as Fig. 3, fixed 1 hour and 5 minutes after insemination. Sperm attached to vitelline membrane, no attachment granules, and no fertilization cone. Perforatorium still delicate. Jelly and both polar bodies have formed. Vesicles of egg nucleus as in Fig. 6.

FIG. 5. Egg from control culture 43 minutes after insemination, showing fertilization cone, attachment granules, and sperm with thickened perforatorium.

FIG. 6. Egg from same culture as Fig. 3, fixed 1 hour and 27 minutes after insemination. Jelly and both polar bodies have formed. Sperm, in another section, attached to vitelline membrane. Vesicles of egg nucleus showing chromosome within.

FIG. 7. Egg from same culture as Fig. 3, fixed 1 hour and 36 minutes after insemination. Jelly and both polar bodies formed. Egg vesicles degenerating. Sperm (in another section) attached to vitelline membrane.

FIG. 8. Egg fixed 2 hours and 17 minutes after insemination with sperm removed from male and kept at room temperature for 21 hours. This egg has not succeeded in completing the division of the cytoplasm and the chromosomal vesicles, about 28 on each side, are scattering. Strongly staining granules around the chromosomal vesicles.



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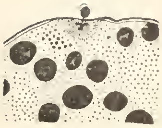

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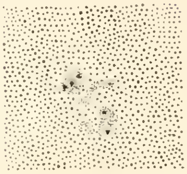

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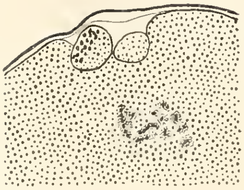

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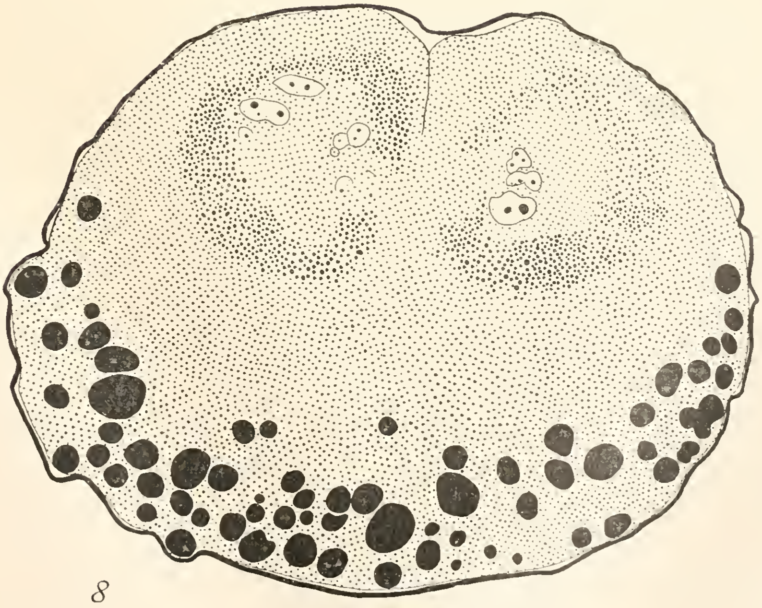

8

NEIL S. DUNGAY.

OBSERVATIONS ON LIVING SOLENOMYA
(VELUM AND BOREALIS).

EDWARD S. MORSE.

The genus *Solenomya*, represented by a few species, is widely distributed throughout the world. It has been found on the east and west coast of North America, in West Africa, the Mediterranean, the Canaries, Australia and New Zealand. The few species agree in almost every detail but vary greatly in size. Our two species, *S. velum* and *S. borealis*, are one inch and three inches long respectively. *S. bartschi* from Manila is eight and one half inches in length. The species vary greatly in the proportional number of individuals. *S. velum* is at times thrown up by thousands on our beaches, *S. borealis* is rarely found. *S. grandis*, which departs more widely from the type than the others, is known by two specimens and a few fragments, while the gigantic *S. bartschi* is represented by a single specimen. The various species have without exception a long semi-cylindrical shell, rounded at both ends, a long and almost straight hinge margin. They all have radiating ribs with an apparent interspace in the series in which however a faint rib may be detected. They all have a highly polished periosteum which runs far beyond the border of the true limy shell, and to which the mantle with its curious system of muscles is closely adherent. The radiating ribs are continued through the overlapping periosteum by a marked thinning of the substance and Professor Drew discovered that when the periosteum folds inward as the valves close these thin areas fold in a plaited manner. In the dry state these interspaces often split and the substance being very brittle it is hard to preserve unbroken in cabinet specimens. The various species vary from a light yellowish brown to a dark brown and even a tar color. The shell within is grayish blue or lead color in *S. borealis*, purplish white in *S. velum*.

Pelseneer[8] has shown that *Solenomya* has a very primitive form of gill and Mitsukuri[7] had previously studied the gill of

Nucula and *Yoldia* and showed also the primitive condition of the gills in these genera. Pelseneer has united *Solenomya*, *Nucula*, *Leda* and *Yoldia* in an order under the name of Protobranchia, forming the lowest order in Lamellibranchs. It is interesting to observe that, while other genera of this low order vary greatly among themselves and from each other, *Solenomya* remains fixed in character despite the wide variation in size among the species and their wide distribution in space.

With the exception of the incomparable work of Deshayes,[2] that of Stempell[11] and Pelseneer[8] on *Solenomya togata* and that of Drew[3] on *Solenomya velum* there have been very few observations on the anatomy or habits of the living creature, and most of the records are derived from accounts of previous observers, and these were in many cases incorrect. Gould says the foot of the animal protrudes behind, whereas it protrudes in front; that the edge of the mantle opening is fringed and that two of the fibrils are larger than the others, which is incorrect, as regards our species and that the palpi are triangular, whereas they are long, narrow and semi-tubular. Stimpson's description, though brief, is without error. Professor George N. Perkins,[9] in his "Molluscan Fauna of New Haven," a memoir teeming with details concerning the soft parts of Mollusca and their habits, has given one of the best accounts of *Solenomya* ever published in this country. Carpenter, in his lectures on Mollusca, says the mantle is closed in front, whereas it is wide open, and that there is a tail on each side of the external opening. Cooke mentions the two long tentacles springing from the siphonal orifice. Stempell figures in *S. togata* two papillæ much longer than the other siphonal processes.

There is a puzzling discrepancy in the figures of the siphonal tentacles of *Solenomya togata* as portrayed by various authors. Philippi[10] figures *S. togata* with short siphonal papillæ resembling a rosette. Pelseneer,[8] in his contributions to the study of Lamellibranchs, figures this species without a trace of long siphonal tentacles. In his introduction to the "Study of Mollusca" he gives a figure of *S. togata* with a pair of siphonal tentacles 17 mm. long. This figure is however copied from Deshayes'[2] "Natural History of Mollusca." It is probably from this figure that Carpenter, Cooke and others have derived the long tail or tentacle mentioned by them. The excessive length of the two tentacles figured by Deshayes suggests the curious retractile tentacle in *Yoldia limatula* as described by Brooks.[1] A careful examination of *S. velum* however showed no trace of such a sense organ. Stempell,[11] who studied this species at Dohrn's laboratory and published an account in 1899, figures two siphonal tentacles 3 mm. in length. Are these differences the result of variation? Is there more than one species of *Solenomya* in the Mediterranean? Have the drawings been made from alcoholic specimens? The mystery can only be cleared up by a study of the living creature.

In the following observations of *Solenomya velum* I have been greatly indebted to Mr. William F. Clapp, of Cambridge, and Miss Marjorie Newell, of Gloucester, for the material herein described. Mr. Clapp brought me thirty-nine living specimens of *S. velum*, young and nearly full grown. These were collected April 27, on Round Flat, a sandy mud area in Duxbury Bay. They were all buried from six to nine inches below the surface. Mr. Clapp believed they were simply inhabiting abandoned worm holes. Although powerful diggers he thinks they ordinarily dig but an inch or two deep. If they do dig to the depth he found them he believes they must occupy the same burrow for a considerable length of time, for in every case he observed the sides of the hole were discolored, closely resembling worm holes. I placed three specimens in a jar of sandy mud and they soon burrowed to the bottom of the vessel, leaving three sharply defined round holes on the surface of the mud. Drew says: "*Solenomya* lives in rather hard mud, frequently very sandy mud, and, I think, keeps its burrow more or less open." The holes which Mr. Clapp observed and which he cautiously suggested might be abandoned worm holes were undoubtedly holes made by *Solenomya*. Verrill says in regard to *S. velum* that it is "occasionally found burrowing in the pure fine silicious sand near low-water mark, about two inches below the surface, but its proper home is in shallow water beyond low-water mark, and it is perhaps most abundant when there is mud mixed with sand and it also lives in soft mud."

The manner of burrowing is peculiar. In one case an individual buried itself siphon end downward, and for three days remained in this position with its disk-like foot level with the surface of the mud. In every case the individual rested on the bottom of the dish, ventral region uppermost and valves widely open, limited only by the closed portion of the mantle which is drawn tense by the distended valves. When placed on the surface of sand or mud it soon pushes itself backward by means of its foot which, assuming a pointed tongue-like shape, is thrust forward and downward lifting the anterior end and thus depressing the posterior end. Others buried themselves and went to the bottom of the dish, where they remained in a horizontal position. Whether they went head first or tail first was not observed. Others buried themselves in the mud and remained out of sight for hours with no burrows communicating with the surface. Drew states that *Nucula delphinodonta* buries itself in the mud with no surface communication. The strong alternate movements of the sides of the siphonal area may be related to the habit of burying itself posterior end downward. As a further proof that *Solenomya* buries itself siphon end downward may be cited the condition of a young *Solenomya borealis* preserved in alcohol for dissection. I found adhering to the anterior end of the shell close to the margin three colonies of a species of sponge. Here was an evidence that the creature had not only been buried siphon end downward, but that the anterior end had been slightly protruded above the level of the sand and had remained in that position long enough for the accumulation of foreign growth. The periosteum is so exceedingly smooth and polished, and the creature is so active in its swimming habits that the adhesion of foreign growth would hardly be looked for.[1] An examination of a number of shells showed little evidence of wear or burial at either end. The pedal opening is so large and the activities of the foot are so incessant that more water is admitted anteriorly than posteriorly and the siphonal opening has little to do so far as conveying water and food to the gill cavity is concerned.

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[1] Dr. Drew informs me that in digging this species from the mud many of them float on the surface of the water, the periosteum repelling the water as if oiled.

If burrowing posterior end downward is a common habit of *Solenomya* it forms an exception to all lamellibranchs that burrow. So far as I know all lamellibranchs that burrow in sand, mud, wood or stone penetrate the substance anterior end first, and in that position they rest. Indeed the foot is the primary instrument used in effecting this penetration. *Solenomya* is unique in this respect.

As before stated the creature, resting on its back, thrusts out its foot in a pointed shape, presses the bottom of the dish, then immediately withdrawing it at the same time expands the fimbriated disk which unfolds in a graceful manner, and in that expanded condition swings back and forth a few times in the pedal opening which is widely distended. The overlapping periosteum then folds abruptly within the shell, which closes at the same time as the thick foot is drawn in between the polished walls of the periosteum. The creature then falls over on its side and remains in that position until the valves again open which is almost immediately. The method of swimming has been accurately described by Drew. The act consists in thrusting out the foot, promptly expanding it and then suddenly withdrawing it, at the same time closing the shell and expelling the water from the siphonal end. These motions are often repeated a number of times without the animal moving at all. When these movements are made with sufficient vigor, however, the animal seems to leap or dart in the water, anterior end forward, going three or four times the length of the shell at each leap.

Stimpson says: "The thinness of the shell enables the animal to make surprising leaps and I have seen it leaping or swimming about the water for some time without touching the bottom. The leap is performed by suddenly drawing in the umbrella-shaped foot, at the same time that water is expelled from the posterior opening by the closing of the valves." I counted the number of darts made by different individuals and, though the specimens I had were probably enfeebled by their long transportation in a small glass vessel, I found the following result—22, 24, 31 and 36 darts respectively were made before the creature fell to the bottom of the dish. The 36 darts were made by a young individual. The darts were vigorous, and were made at the rate of 90 to 100 a minute.

The violent activity of the animal in its rapid darting through the water, the repeated thrusting out of the heavy pedunculate foot and vigorous closing of the valves accounts for the necessity of the continued absorption of food as indicated by the rapid accumulation of flocculent fœcal matter in the dish.

The thirty-nine specimens, young and nearly adult, collected April 27, were placed in a white enamelled pan and showed no signs of increased enfeeblement for a week or more. The water was changed often. At the end of three weeks they were all dead, the young ones surviving the longest.

The individuals showed no sensitiveness to the obstruction of light, nor did they align themselves in any special way in relation to the lighted window. They did not seem to have the sensitiveness of other lamellibranchs; the jarring of the table did not cause them to close, though agitating the water or touching them with a needle ever so slightly prompted them to close their shells, which remained closed for a few seconds, when they slowly opened and repeatedly thrust out the foot. In fact they became very lively after agitating the water.

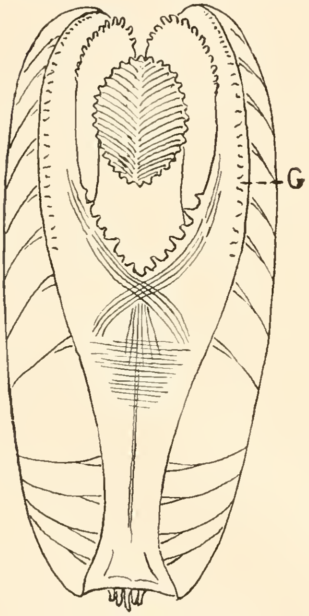

Fig. 1. View of animal from below.

As one views a specimen from below with the valves wide open and the closed part of the mantle stretched like a drumhead from the margin of the periosteum, the calcified portion of the shell is hardly in view. The periosteum, extended as it is when the valves are open, shows no marked shoulder or channel at its junction with the thin membranous mantle, though in section a slight break is seen. The pedal opening is wide and extends backward nearly to the center of the body, where the free mantle edges come together, forming an elongated oval opening when distended, and through this opening the thick stocky foot is seen occupying the whole space (Fig. 1). At the point of junction of the two sides of the pedal opening there are crowded together muscle fibers, producing a silvery appearance, and at this point muscle fibers that run parallel to the borders of the pedal opening—which Drew described as in the nature of sphincter muscles—cross to opposite sides and continue their course posteriorly. May not the origin of the peculiar sharply defined cruciform muscles in *Solecurtus*, *Tagelus*, *Macoma* and *Tellina* be traced to these separate crossing muscle fibers in some primitive lamellibranch like *Solenomya?*

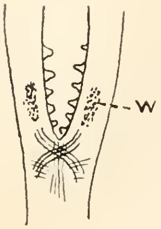

FIG. 2.

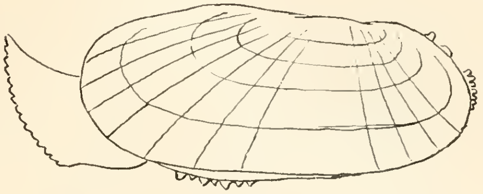

FIG. 3.

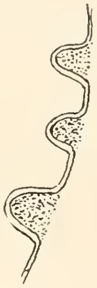

FIG. 4.

FIG. 2. Pedal opening showing white area, W.
FIG. 3. Side view of animal.
FIG. 4. Papillæ on margin of pedal opening.

The edge of the pedal opening is at times reflected and at its junction in the median line a few blunt papillæ or tubercles appear along the edges to nearly half the length of the opening. These are so aligned that when the two edges come together in closing the tubercles interlock. In Fig. 2 the tubercles are seen with the pedal opening nearly closed. They vary in size, are contractile and stand out at a sharp angle from the border so that they are distinctly seen when the animal is viewed from the side (Fig. 3). Until nearly adult these tubercles are translucent, but in the oldest specimens they become opaque white, resembling white kid, and appear hard and horny until touched when they partly retract. They are covered with a transparent epithelium (Fig. 4). This peculiar whitening not only covers the tubercles but whitens the mantle border from which they spring. A blotch of white is also seen between the tubercles and the keen edge of the periosteum, which at this point is also touched with white. Even the base of the foot behind has a small blotch of white showing only when the foot is greatly elongated. This coloration in all exposed parts of this region is certainly a curious feature. It is as sharply defined and localized as if one with a paint brush had delicately touched with white every exposed part of the animal within this limited area. It is true the external shell is also touched with white at the anterior end. In the young a dot of white is seen in the first three or four transparent interspaces of the free periosteum. In older specimens these white spots are drawn out in broken lines; not in all specimens, however. In older specimens anteriorly the whitening is sometimes seen on the dorsal side of each rib. This peculiar coloration is apparently correlated with the attitude of the animal in nature. Resting on its back the ventral region of the animal shows this whitening. Are the white markings at the anterior end of the shell another indication that the creature more often or habitually buries itself siphonal end downward?

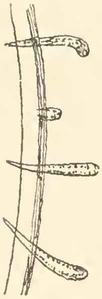

FIG. 5.

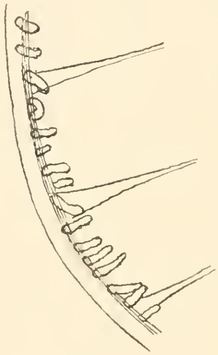

FIG. 6.

FIG. 5. Glands from side of pedal opening.
FIG. 6. Glands from anterior mantle margin.

Along the anterior half of the mantle, as it springs from the edge of the periosteum, are seen a number of what appear to be short white lines with glandular enlargements. They begin a little in advance of where the mantle parts, forming the pedal orifice, and are separated by a considerable space (Fig. 5), becoming crowded toward the rounded anterior border (Fig. 6). They spring from just under the edge of the periosteum and point in the general direction of the median line, but are disposed irregularly in the membrane, some inclined forward and others backward, but most of them are at right angles to the mantle border (Fig. 1, G). Under a higher power these bodies have the appearance of setigerous follicles. Stempell figures one in his memoir of *S. togata* as a simple alveolar border gland. His figure shows a much more bulbous gland and the thread-like extension is not shown as in those here figured (Fig. 5). It was some time before I determined that there was no protuberance beyond the surface of the mantle, so strong was their resemblance to setigerous follicles.

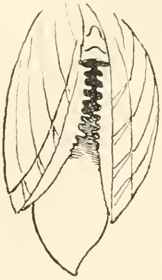

FIG. 7.

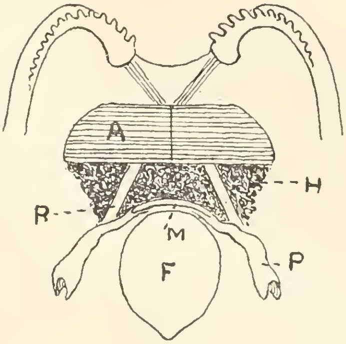

FIG. 8.

FIG. 7. Anterior view of animal.

FIG. 8. Anterior end from below: *A*, anterior adductor muscle; *F*, foot in section; *H*, hepatic follicles; *M*, mouth; *P*, palp-appendages; *R*, pedal retractors.

The margin of the mantle becomes thickened as it borders the anterior end of the shell and ends abruptly just before the shells meet dorsally. Just in front of where the shells meet above are two short unpaired tentacles or tubercles in the median line and then ten or more on the edge of the rounded anterior edge of the mantle on each side diminishing in size, however, toward the ventral region, where they cease (Fig. 7). Writers have described the whole free edge of the mantle as bearing these papillæ and even shown them in figures. Looking at this region from below the abrupt termination of the thickened mantle margin and the papillæ are shown (Fig. 8). A triangular muscular layer seems to connect the two thickened portions of the mantle dorsally. Also a narrow band of fibers runs from the abrupt terminations of the mantle converging centrally. Fig. 8 shows the relation of these various parts in front of the anterior adductor.

As the animal becomes enfeebled the mantle (or, what would be a more proper name, the ventral membrane) ruptures, exposing the dark-colored gills below. There is a median suture in this ventral membrane and in one rupture the suture became dislocated, showing there was a strain upon it. In another case a small rupture appeared on each side of the median suture. That this membrane limits the expansion of the valves is shown by cutting the membrane, when the valves immediately open wider in much the same way as when the adductors are severed in other lamellibranchs. Fig. 9 represents the various ruptures, and at the same time illustrates different attitudes of the foot. In the adult the pedal opening extends back nearly half way to the center of the body. In the young it extends a little over a third back. Viewing the shell from above, the exposed ligament presents some curious features which I have tried to represent in Fig. 10. In the vicinity of the umbones a narrow elongated wedge-shaped substance, black in color, is seen, behind which a brown oval ligament appears, split at its posterior end in which is wedged a white substance. From the junction of the two valves anteriorly a narrow wedge-shaped substance narrowing posteriorly is peculiar in being a polished white. In the young

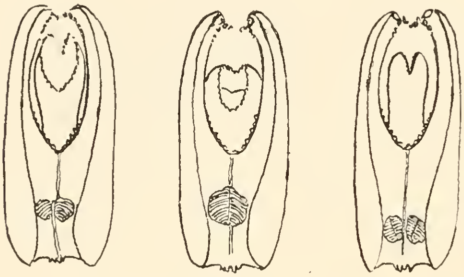

FIG. 9.

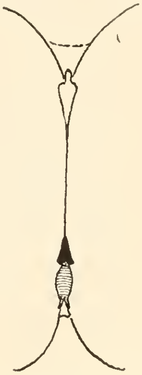

FIG. 10.

FIG. 9. Various attitudes of foot, and ruptures in ventral membrane.
FIG. 10. Dorsal view of hinge margin.

the periosteum is light yellowish, growing darker as age advances. The hypobranchial gland appears as a mass of globular cells containing short rod-like bodies. A number of graceful attitudes are shown in the movements of the pedal disk which I found difficult to figure. The fimbriated edge shows as distinct short tentacles with grooves running down outside, corresponding to the tentacles and corresponding lines or grooves in the disk running to the median line resembling a diminutive actinoid coral *Fungia*. At other times the edge of the disk appears serrated. These number thirty in all (Fig. 11). In the young these resemble long papillæ.

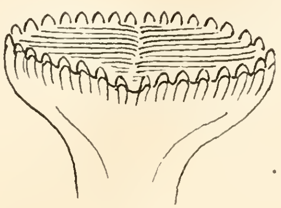

FIG. 11.

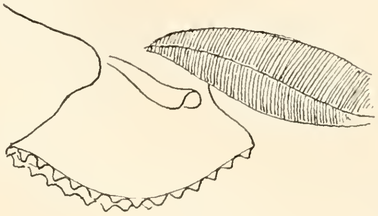

FIG. 12.

FIG. 11. Dorsal view of pedal disk.
FIG. 12. Side view of foot, showing palp-appendage and branchia.

The gills are most peculiar in *Solenomya*. As Pelseneer has shown, there is only one gill on each side having an upward and downward series from a central base. This line dividing the upper and lower series runs from the dorsal anterior portion of the gills to nearly a ventral point posteriorly (Fig. 12). The filaments are distinctly separate and are beautiful in their regularity. The filaments are arranged on the support from which they spring directly opposite each other. Each filament is supported by a chitinous rod in one series, slightly bent at the end—firm yet elastic. The same chitinous framework is seen in the gills of *Nucula proxima* and *Yoldia limatula*, as shown by Mitsukuri, Drew and others. Mitsukuri,[7] in describing the chitinous framework supporting the gills in *Nucula proxima*, says: "Whether it is really formed of chitin I do not know, but as previous writers have described the substance as of that nature it will be convenient to use the term 'chitinous support' for the present." The gills are colored a light brownish purple, their posterior terminations are free and lighter colored (Fig. 13).

A view of the posterior end of the animal reveals a single long siphonal opening which often changes its outline, at times being an oblong, oval slit, but usually the upper part, corresponding to the anal opening, is much larger, giving the aperture a double-gourd-shaped form, the upper bulb being larger. Above the anal region, just where the two shells meet, is an unpaired tubercle, or tentacle which is contractile. Stempell figures a similar tubercle in *S. togata*, and in another form to be described later a very long tentacle is seen. Just below this tentacle a pair of blunt tubercles arise which are very contractile and appear at times as two and even three pairs of tubercles. The sides of the anal opening are without tubercles. The narrow branchial opening is bordered on each side by from six to eight short tubercles or papillæ hardly varying in size. These are also contractile. A few attitudes of the siphonal opening are shown in Fig. 14.

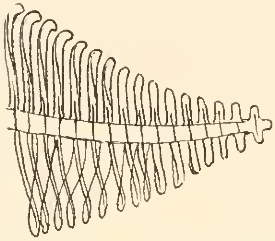

FIG. 13. Termination of branchia.

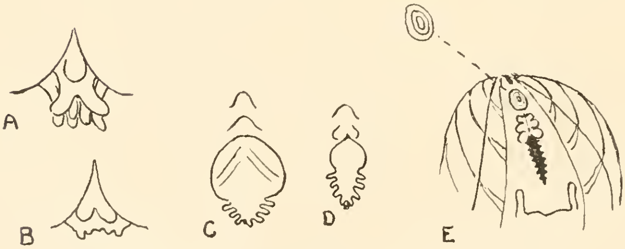

FIG. 14. Siphonal opening: *A*, *B*, from above; *C*, *D*, from behind; *E*, from behind at an angle.

A view of the siphonal opening from the side shows no protrusion as in the siphonal expansion of siphonated lamellibranchs. Indeed there is no appearance of a siphon, simply an opening with papillæ about it and these quite unlike the long pointed papillæ of the siphonated lamellibranchs or even the truncate papillæ of *Saxicava*, though morphologically they are the same. On viewing this region from above the papillæ appear bunched together or spread apart, the result of the sudden dilatation or partial contraction of the opening.

The sides of the siphonal opening are in constant motion in and out, though this motion is alternate and rhythmic. The proboscidiform foot swings from one side to the other within the shell, like an elephant's trunk and this motion may be related to the alternate siphonal movements; this correlation, however, was not observed.

Just below the siphonal opening a slight depression is seen on the mantle on each side connected by a transverse depression forming a symmetrical figure. Stempell shows a marking of the same nature in *S. togata* in the form of an angle with the apex downward.

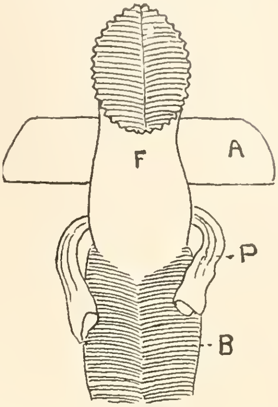

FIG. 15. Anterior end from below: *A*, anterior adductor muscle; *B*, branchia; *F*, foot; *P*, palp-appendages resting on branchia.

A view of the animal as it rests in the usual position on its back with valves widely apart will reveal the attitude and behavior of the palpi (Fig. 15). Cutting the ventral membrane will give a clearer view of these parts. The palpi are adherent for a short distance to the side of the foot but are free beyond, and extend backward and downward. These organs are long, slender, semi-tubular with ends much larger and the whole structure delicate and diaphanous. No line or angularity in section indicates in any way that this semi-tubular appendage has come about by the adhesion of two palpi. The palpi of other lamellibranchs are more or less triangular in shape with their upper edges partly united. In *Solenomya velum*, the long tube-like appendages encircle the base of the foot with their dilated extremities resting directly on the compact sides of the gill plates, and in this position are in gentle and constant motion with occasional side jerks. After watching these browsing movements for awhile one becomes convinced that the behavior of the ends of the palpi is that of feeding. Even the peculiar jerks at times suggest that some larger morsel has been caught. The protean shapes the appendage assumes are shown in Fig. 16. The strong ciliary action of the gills is continually sweeping along particles of organic matter toward the feeding appendages which gather the stuff and convey it by ciliary action to the mouth. The movements of these appendages may be distinctly seen through the ventral membrane and at no time are they at rest. The continual ingestion of food is indicated by the great quantity of excreta which is voided. In this connection it is interesting to note that Drew[6] in his memoir on the life habits of *Yoldia limatula*, another member of the Protobranchia, describes long appendages to the palpi which extend backward beyond the posterior end of the shell and rest on the sand. These appendages are semi-tubular and being ciliated "rapidly elevate the mud which is full of living organisms and finally pass it between the palpi," and thence, of course, to the mouth.

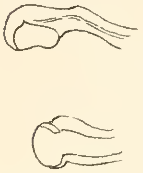

FIG. 16. Ends of palp appendages.

Mitsukuri, having described the palpi in *Nucula proxima*, says: "At their posterior end there are two remarkable structures. One of these is a hood-like structure which is the posterior prolongation of the united upper edges of the inner and outer palpi. The other, lying immediately below the first, is a long tentacular appendage. It is a hollow tube, open, however, along a line on its posterior aspect, and having its cavity continuous with the space between the two palpi. As it has been seen protruded with the foot outside of the shell, and since, in alcoholic specimens, a great deal of dirt and sand is found along its length and between the palpi from its base to the mouth, it is no doubt a food-procuring organ, probably sending a constant stream of nutritive matter to the mouth by means of its cilia. It is interesting to notice in connection with this appendage that in *Nucula*, the gills, unlike those of ordinary lamellibranchs, must be practically useless for obtaining food, as will be evident from the following description of them," etc. Drew[4] finds these palp-appendages in *Nucula delphinodonta* functioning as food collectors. The creature is immersed in mud out of sight and only when placed in shallow mud was he able to observe its behavior. The gills are certainly very small in *Nucula*. In *Solenomya*, however, the gills are very bulky, filling nearly half the mantle cavity, are highly ciliated and as we have seen the palp-appendages rest directly on their anterior surfaces. There can be no question that these appendages in *Solenomya* are strictly homologous with the palp-appendages in *Yoldia*, *Nucula* and allies. The gills, however, are the food accumulators from which as we have seen the palp-appendages, collect the material for nutrition. The mouth is difficult to make out in the living creature. In two instances I have observed a slightly brownish line marking the position of the mouth.

While there are many features in common between *Solenomya* and the other members of the order Nuculidæ as seen in the posterior position of the umbones, the primitive gills, the palp-appendages, the fimbriated disk-like foot, the highly polished periosteum; the absence of true palpi in *Solenomya* might be considered an evidence that this peculiar form stands lowest in the order Protobranchia.

Some years ago Miss Newell discovered at Annisquam a colony of what was supposed to be *Solenomya velum*. A few were brought home alive, but circumstances were not favorable at the time for detailed study and only a few drawings were made. The shell is apparently identical with that of *S. velum* herein described, yet the character of the siphonal opening or rather the appendages surrounding that aperture are widely different. The single siphonal orifice is ano-branchial as in the other species of *Solenomya*. Above this orifice is a very long unpaired tentacle with broad base. On each side of the anal region is a broad flap bearing five tentacles, the lowest one small, the next one long slightly curved upward with three minor twigs springing from its side, the next three smaller and diminishing in size. Below this process is a wider flap supporting nine tentacles of nearly uniform length. Between the anal and branchial processes viewed from the side is a white band! I hesitate giving these details for fear some systematist will instantly announce a new genus or family. Nevertheless, no figure nor description of *Solenomya* yet published has shown anything approaching these remarkable processes. The unpaired dorsal tentacle is short in *S. togata* (Fig. 18), even shorter in *S. velum* (Fig. 19), while in this form (Fig. 17) it is exceedingly large with a broad base. The pedal opening shows the same tentacles starting from the posterior end of the opening. The same alternate movements of the siphonal opening were observed. The shell as before remarked differed in no respect from that of *S. velum*, and I am puzzled at the remarkable difference in structure of the siphonal opening. The question arises whether the Annisquam form may not be the young of *S. borealis*. This can only be decided by securing a living specimen of this rare form.

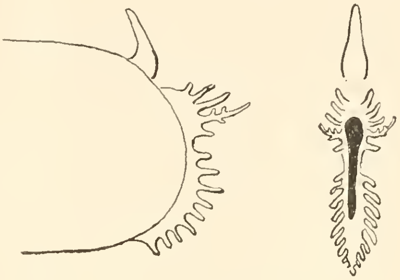
FIG. 17. *Solenomya* sp.? Annisquam.

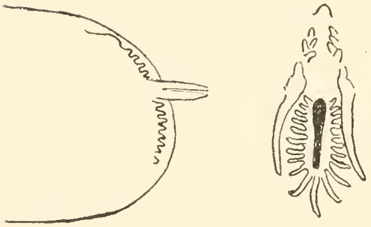
FIG. 18. *Solenomya togata.*

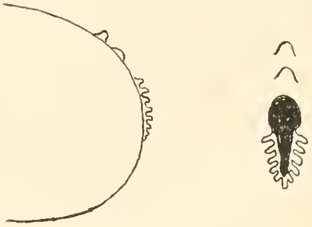
FIG. 19. *Solenomya velum.*

Professor Drew,[5] in his observations on the habits, anatomy and embryology of members of the Protobranchia, alludes to the great diversity in structure and concludes that the protobranchia have been derived from a primitive type. The resemblance he finds in certain stages of the embryo of *Yoldia* and

Nucula to those of certain other mollusks is interesting in the fact that these mollusks are in every case low and aberrant forms. He says: "The most striking peculiarities in the development are connected with the formation and disappearance of the tests. Outside of the group, so far as I have been able to learn, *Dondersia* is the only other mollusk whose embryos are known to be provided with similar tests."

Dondersia is now regarded as belonging to the Amphineura, but for awhile was looked upon as related to the worms. Drew remarks that the young embryos of *Dentalium* bear certain resemblances to the embryos of *Dondersia*, *Yoldia* and *Nucula*. Here again a resemblance is seen to an aberrant group of mollusks whose affinities were for a long time obscure. Drew also says that a somewhat similar resemblance is noticeable in the case of the embryo of *Patella*. Again a resemblance is found to a group whose characters are archaic. Dall, in speaking of the Docoglossa, says the various forms manifest what may be termed a peculiar persistency of immaturity when compared with other groups of gasteropods. Korschelt, Patten, Fisher, Lankaster, Pelseneer and others testify to the primitive characters of *Patella*. In my paper on "An Early Stage of *Acmæa*," I have collected a number of extracts from the above authors in regard to these low characters.

Pelseneer agrees with Stempell that the characteristic features of *Solenomya* represent the oldest living group of the lamellibranchs. He calls attention to the absence of a protractor pedis, extensive overgrowth of ventral mantle edges, peculiar development of the branchi-anal siphon, the form of shell and absence of interlocking teeth, with position of ligament, remarkable overlapping of periosteum, prismatic structure of limy shell, rudimentary form of mouth lips and mouth appendages, almost without winding of intestines, excessive elongation of the ventricle, the presence of pericardial glands, position, size and structure of gills, separation of kidneys without cross communication.

Interesting features will doubtless be revealed when the embryology of *Solenomya* shall have been made known, and with the abundance of one species on our coast, *S. velum*, we hope before long light may be thrown on the subject.

APPENDIX—NOTES ON LIVING *Solenomya borealis.*

Since the above observations were made on *Solenomya velum*, and the matter in type, the opportunity has been given me of studying living specimens of the large species *Solenomya borealis.* My friend, Major John M. Gould, collected a number of living specimens of various sizes from a young one, measuring 9 mm., to full-grown individuals, measuring 83 mm. These were obtained from dredgings in six to seven fathoms of water by a huge dredging machine engaged in deepening a channel in Portland harbor, Maine. The work of collecting, as may be imagined, was done under desperate conditions. Jumping in between avalanches of mud and water, glancing, raking and jumping out again! In this rapid reconnaisance Mr. Gould observed that the creatures were buried in holes and got the impression that they were buried posterior end downward, the broad, light-colored disk-shaped foot projecting from the holes. This attitude, as before remarked with regard to *S. velum,* is contrary to the behavior of all lamellibranches that bury themselves wholly or partially. I am still in doubt about the matter. It is interesting to note, however, that when the creature was placed on coarse material it behaved in precisely the same way as *S. velum.* In every case resting on its back it thrust out its foot, raised the anterior end of the body as if endeavoring to thrust the posterior end into the mud. In this connection, it is interesting to observe that the anterior ends of the shells of older specimens were covered with films of slime while the posterior ends were clean and polished.

In only one instance has the animal been seen to swim or dart through the water though both young and old were specially observed for this behavior. The foot was often thrust out as in *S. velum*, but not so often nor with such energy. No lateral swing of the foot was observed. The periosteum appeared oily as in *S. velum* and repelled the water, and Dr. Drew informed me that this feature was so marked in *S. velum* that when he dug them from the mud they sometimes floated on the water.

The character of the siphonal end upon which marked specific differences will be established was quite different from *S. velum* and it can now be positively stated that *S. velum* and *S. borealis* are distinct species. It is surprising, however, to see how closely they resemble one another (Fig. 20). At first sight *S. borealis* seemed to be an enlarged *S. velum*. The color of the foot and ventral membrane varies. In *S. borealis* the foot is a light brown with a tinge of purple, darker on the ventral keel, with a darker blotch near the ventral portion. The papillæ on the edge of the pedal disk were longer and sharper than in *S. velum* though the same in number. The tubercles on the edge of the mantle opening were the same in number and position, standing out at right angles from the ventral membrane. Their color, like the membrane, was brownish, the tips of the tubercles being dark brown. No trace of a white pigment-like color on ventral membrane or tubercles was observed in either young or old. The palpi were not so long though the ends were more foliated (Fig. 22). Their movements were not so active. The palpi rested on the anterior portion of the gills and were evidently engaged in secur-

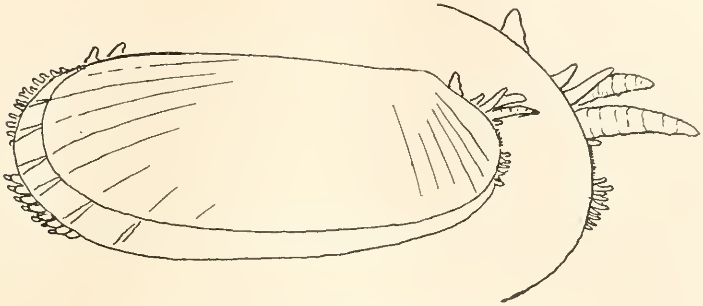

FIG. 20. Side view of *S. borealis*, natural size.

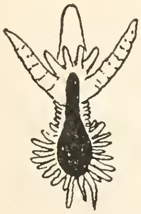

FIG. 21.

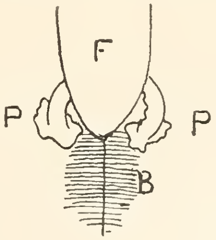

FIG. 22.

FIG. 21. Siphonal opening.

FIG. 22. Anterior end from below: *F*, foot; *P*, palp-appendages resting on the branchia; *B*, branchia.

ing food as described in *S. velum*. Alternate movements of the side of the siphonal opening were observed as in *S. velum*. The anterior aspect of the creature was much the same, the two median dorsal tubercles, one behind the other, and the anterior edge of the mantle bearing papillæ. The posterior aspect was quite different from *S. velum* and resembled more closely *S. togata* of the Mediterranean as figured by Stempell. The resemblances are seen in the large size of the median dorsal tubercle, the few small tentacles in pairs, succeeded by a pair of very long tentacles, then a number of very minute tentacles bordering the upper edges of the siphonal opening followed by a number of tentacles increasing in size to the lower portion of the opening (Fig. 21). All of these tentacles were retractile, the upper series appearing like round tubercles when contracted. The longer tentacles surrounding the orifice were in constant action, a bending-in movement as if grasping.

In the collection of fifteen specimens of *S. borealis* the individuals varied in length from 9 mm. to 83 mm. Omitting the smallest specimen the others were easily arranged in four distinct series of sizes as follows:

1st Series.	2d Series.	3d Series.	Full Grown.
24 mm.	39	57	73
29 mm.	40	59	77
31 mm.	44		77
31 mm.			78
			83

Twenty-eight specimens of *S. velum* from Sandwich were in the same way readily grouped in series. One measured 7 mm. in length, 6 averaged 10 mm., 8 averaged 12 mm., 5 averaged 13 mm., 6 averaged 15 mm. and 3 averaged 19 mm. From this one might infer that *Solenomya* is not an annual.

In conclusion I wish to say that this brief examination of the external features of *S. borealis* has convinced me that my supposition that the Annisquam individual might be the young of *S. borealis* is incorrect. Furthermore that a study of a more advanced specimen of *S. velum* at Woods Hole showed a nearer approach to the Annisquam specimen. I have never seen a minute specimen of *S. velum* and Professor Drew informs me that he has never found one with eggs. The protoconch of *Solenomya* will show interesting features and the embryology of this low form will be of great importance.

Explanation of Figures.

Note.—Only the largest specimens averaging 19 mm. were studied. The various degrees of magnification of the figures here given may be understood in a general way by realizing that the entire length of the animal was 19 mm.

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A CHEMICAL SIGN OF LIFE.[1]

(From the Laboratory of Biochemistry and Pharmacology, The University of Chicago; and Marine Biological Laboratory, Woods Hole, Mass.)

SHIRO TASHIRO.

From the earliest time to the present, our criterion of life has been connected with the changes which are brought about by death. The locomotive power of living matter is one of the first things that disappears when it dies. Thus the idea of movement erroneously led the simple minds of our ancestors to believe that wind, fire, thunder and water had life. By close study of the locomotion of living matter, however, we have gradually traced this part of living phenomena to irritability of tissue—the property characteristic to living tissues only. This protoplasmic irritability is not only the potential of living matter, to give rise to physical changes when a stimulus is applied to it, but the fundamental and the only characteristic which is common to all the living as long as they possess the power to perform their own functions. And it is this property, according to Professor Mathews,[2] that not only is the most probable point of attack of natural selection but is also one of the main factors which determines phylogenetic development of organisms.

The presence of this irritable property in tissue—the universal sign of life—cannot easily be determined in all living tissues. The most common way of determining irritability is the physical changes brought about by a stimulus. This physical change, however, is not an unfailing indication of a response of the tissue against stimulation. Several tissues in the animal, such as nervous tissues, and an abundance of examples in the plant kingdom, do not manifest at all any visible change, when stimulated. Not only those living do not show such mechanical changes while functioning, but several non-living matters constantly undergo physical changes when disturbed by external conditions. Therefore it has been customary for the biologist to decide whether or not matter is living by several other accompanying changes during the performance of a function. Such changes have been measured by rise of temperature, production of CO_2, histological variation before and after the stimulation, and electrical response. None of these functional changes when taken individually can be considered as a specific sign of life.

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[1] I wish to thank Dr. F. R. Lillie, through whose kindness I was occupying a table in the Marine Biological Laboratory, Woods Hole, Mass., at the time when the apparatus for these experiments was made.

[2] Mathews, *Amer. Naturalist*, XLVII., p. 94, 1913.

Although Herzen claims that under certain conditions of local narcosis, the nerve fiber may give an action current although no muscular contraction follows, and O. B. Ellison recently demonstrated by the use of cinchonamine hydrochloride the absence of negative variation without abolishing excitability, yet according to Waller the presence of life can be demonstrated by an electrical change. In his admirable book on the "Signs of Life,"[1] he states that chemical change is a sign of life and an electrical change is a sign of a chemical change; therefore the electrical change is the sign of life. It is very interesting to note in the case of a dry seed, he could measure equally well quantitatively the different electrical changes according to the different ages which characterize the different degrees of vitality of the seed, in spite of the fact that he could not detect any chemical change which usually produces CO_2. He concludes, however, that it is possible, or rather certain that our method of chemical investigation is not refined enough to reveal to us the smallest and most infinitesimal change that may be going on in apparently dry or dormant seed. It was this conclusion of his that suggested to me the desire to make an inquiry to ascertain whether or not I could find some new method by which an easy sign of life may be observed.

When I had constructed a new apparatus,[2] which can detect CO_2 as small as 0.0000001 gram, the measurement of irritability became much simpler, for with it we established a few new facts which deal with the fundamental nature of protoplasmic irritability. The idea that irritability in general is closely associated with chemical phenomena has been set forth long ago. Professor Mathews, in his paper on "Animal Oxidation," expressed the idea that cellular respiration must be the fundamental process of all organic activity around which all functional phenomena are intimately connected. Later in his study on the action of ether and other anæsthesias on an anaerobic tissue he confirmed his idea that all the substances that affect irritability must necessarily attack the tissue respiration first. With the aid of the new apparatus, the direct evidence for his hypothesis has been brought forth in connection with the study of metabolism of the nerve fiber, in which three fundamentally interesting facts have been established. In the first place, the most excitable tissue of all the protoplasm, the nerve fiber, is constantly undergoing chemical changes, giving off CO_2. In the second place, when this tissue is stimulated CO_2 production is greatly accelerated, giving more than double the amount. Finally, the rate of CO_2 is greatly influenced by conditions such as anæsthesia, which are known to affect tissue irritability, showing a direct relation between respiration and excitability.[1]

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[1] Waller, "Signs of Life," New York.

[2] *Am. J. of Physiol.*, XXXII, p. 137, 1913.

Before I had concluded, from these facts, that all the irritable tissues must respire and should give off more CO_2 when stimulated, a crucial experiment was done on a dry seed. Waller's conclusion has been fully realized. A living dry seed not only gives off CO_2 quantitatively proportional to its weight, but also gives off more CO_2 when stimulated, a phenomenon true to living seeds only. These facts enable me to propose a new sign of life, namely, a chemical sign. The criterion is simple. If we are given a tissue which gives more CO_2 when stimulated, the tissue must be alive; it is excitable. A discovery of a remarkably simple method of stimulation made this chemical sign of life much more easily practicable to all living tissues. Simple mechanical crushing of the living tissue is the new method. I have already argued elsewhere[2] that the phenomenon that the nerve gives off more CO_2 when crushed, is due mainly to an extreme stimulation and that it is characteristic of living, excitable tissues only. Therefore without any attempt to settle the question as to how CO_2 production is increased, we can use this method as a means to denote protoplasmic irritability, as long as we can abolish this sign of life by rendering the tissue unexcitable. The nerve, dry seeds, including wheat, oats, rice and several other living tissues from animal and plant kingdoms, if treated with ether, or killed, can never be made to produce more CO_2 by crushing, whereas, a tissue however little CO_2 it may produce normally will give off more CO_2 when crushed, provided it is living. From these general findings, I conclude that this method can be used to detect vitality of protoplasm under normal conditions.

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[1] *Am. J. of Physiol.*, XXXII., pp. 107–136, 1913.

[2] *Am. J. of Physiol.*, XXXII., p. 121, 1913.

The details of this simple means of finding the sign of life are as follows:

The biometer is used. This is an apparatus which has two respiratory chambers, each furnished with a cup in which a drop of barium hydrate can be introduced. With it, the comparative outputs of CO_2 as small as 0.0000001 g. from the two tissues can be estimated simultaneously.[1] Two dry living kernels of the same seed, with equal weights, are chosen. One is placed in the right chamber, and the other is crushed and placed in the left. After the necessary cleaning of the apparatus with CO_2-free air, a drop of the barium hydrate is introduced upon each cup in both chambers. By watching the drop, it would become obvious that the crushed seed is giving off more CO_2 than the uninjured, as indicated by the speed of formation and quantity of precipitate of the barium carbonate. Not only such distinction between two can be observed, within a few seconds, in the case of the tissue, which normally gives off a comparatively large quantity of CO_2, but also dead tissue whether or not it gives off CO_2 without crushing, will never produce more CO_2 when mechanically smashed. In other words the phenomenon of production of more CO_2 by crushing is characteristic only of a living tissue. By killing the seeds, and repeating the experiment, such a conclusion can easily be confirmed.

With these facts, I am proposing a new sign of life, namely, a chemical sign of irritability. It is a measurement of CO_2 due to stimulation. It is not exhalation of CO_2 which is characteristic of life, for there are many dead matters which give off CO_2 continuously, but it is the increase of CO_2 production when crushed which is certainly a phenomenon associated with excitable tissue only. Hence, when a tissue gives off more CO_2 when mechanically crushed, or injured, it is a sure sign that this tissue is living.

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[1] The detail of this apparatus is described in *Am. J. of Physiol.*, XXXII., p. 141, 1913.

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BIOLOGICAL BULLETIN

SIZE DIMORPHISM IN ADULT SPERMATOZOA OF ANASA TRISTIS.[1]

E. C. FAUST.

I. INTRODUCTION.

The work on which this paper is based was undertaken with the object of determining the existence or non-existence of size dimorphism in the mature spermatozoa of *Anasa tristis.* Spermatogenesis studies of this form have shown that one half of the spermatozoa receive one more chromosome each than the other half. A corresponding difference in size of the mature spermatozoa may therefore be expected.

The study was carried on during the academic year 1912–1913, under the direction of Professor Charles Zeleny, to whom my thanks are due for his many valuable suggestions.

II. MATERIALS AND METHODS.

1. *General Account.*—The material used in this study was collected from two localities, Urbana, Ill., and Bradford, N. H., during the month of October, 1912. The testes of these individuals were teased out in normal salt solution and made up into temporary and permanent smear preparations.

Smears of adult spermatozoa were prepared by the aceto-carmine method and by the osmic-hæmatoxylin method. In addition *intra vitam* staining was tried, but without success. The first mounts were made up as aceto-carmine preparations. Although this affords an excellent preparation for the study of spermatogenesis, it is not a good method to use in the study of adult spermatozoa. Curling is frequent. Distortion by swelling occurs more than is desirable. In the head of the spermatozoön no *inner chromatic rod* is differentiated from the cytoplasmic envelope. Moreover, it often takes several days to measure a considerable number of individuals, so that temporary preparations, such as those obtained with aceto-carmine, are objectionable. For this same reason *intra vitam* staining was abandoned.

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[1] Contributions from the Zoölogical Laboratory of the University of Illinois, under the direction of Henry B. Ward, No. 23.

For the most of the preparations Delafield's hæmatoxylin was used and proved much more successful than Schneider's aceto-carmine. The testis was taken out of the individual and placed in normal salt solution. It was then transferred to a slide that had been previously coated with a thin film of albumen fixative. The spermatozoa were teased out on the slide and examined in a drop of some fluid, such as Ringer's, to determine their motility or non-motility. After most of the solution had been allowed to evaporate so that only a bit of moisture was left around the spermatozoa, the preparation was fixed in osmic fumes for thirty seconds and then stained in Delafield's hæmatoxylin. This method was found to give a maximum number of straight spermatozoa with an *inner chromatic rod* well differentiated.

The microscope used in the measurements was equipped with a Leitz 2 mm. oil immersion objective and Zeiss compensating oculars. A large number of individuals was measured and a curve of variability plotted. In every individual it was the *length of the inner chromatic rod* which was determined. A description of this chromatic rod occurs later under the description of a mature spermatozoön.

2. *Sources of Error.*—The probable sources of error that need special consideration are (1) imperfect technique in preparations; (2) immaturity of the spermatozoa; or (3) incorrect measurement.

(1) *Error Due to Imperfect Technique in Preparations.*—Several smears of mature spermatozoa were prepared by the aceto-carmine method. Measurements made from them were not uniform, due to faulty technique. Many of the sperm heads had been curled or shrunken in fixation. Lack of an exact tip or base of the chromatic rod could have given rise to an error of from 1μ to 5μ in measurements of length. Distention and distortion were common. On account of the faulty prepara-

tions, the results obtained from them have not been included in the data.

In the aceto-carmine preparations it was found that drawing off the excess stain by a bit of filter paper as recommended, carried along with it many spermatozoa. In fact, preliminary measurements of such preparations showed a prevalence of larger spermatozoa on the side toward which the drawing was being made. Precautions were taken to eliminate this fault by fixing the spermatozoa to the slide with a thin film of albumen fixative. Also osmic fumes were used in preference to a liquid killing agent and Delafield's hæmatoxylin, instead of some more complicated stain, in order that all the spermatozoa might be preserved. This osmic-hæmatoxylin combination has been found particularly effective in producing straight spermatozoa with well-marked chromatic elements. In the preparations from which data were compiled from ninety-five to ninety-seven per cent. of the spermatozoa were sufficiently straight for measurement.

(2) *Error Due to Immaturity.*—When preliminary mounts were made about the first of October, 1912, the material was found to be too immature for the purpose of this work. All stages in spermatogenesis were present from the spermatogonium to the young spermatids, but no adult spermatozoa were found.

One of the problems confronting the writer in determining suitable preparations for study was the recognition of a truly mature spermatozoön. In general, the attenuated character of the head, with the chromatic rod (nuclear element) extending almost its entire length, is sufficient for the recognition of a mature spermatozoön. On that basis all preparations where more than five per cent. of the individuals were immature were discarded. In order to satisfy himself more fully of the maturity of the individuals measured, the author examined all of them in Ringer's solution, which demonstrated their motility. In this fluid the developing spermatids and immature spermatozoa were quiet, while the fully ripe spermatozoa were active for a considerable length of time. However, motility is not to be taken as a general criterion of maturity. In the house-fly, *Musca domestica*, for example, immature forms are motile. Again, these spermatozoa were similar in all respects to those obtained at Woods Hole in August, 1913, from adult males of this species. Because of their structure and specific reaction the author is assured that all spermatozoa entering into consideration in the data are mature individuals.

The description of a mature spermatozoön becomes necessary at this point. As far as the writer has been able to discover no one has described a *chromatic rod* with cytoplasmic sheath for the mature spermatozoön of *Anasa tristis*. Paulmier's description and figures show no such structure. Meek's figure for the "penultimate stage" of maturing spermatozoa of *Stenobothrus viridulus* shows something of the nature of this chromatic rod (Plate III., Fig. 36).

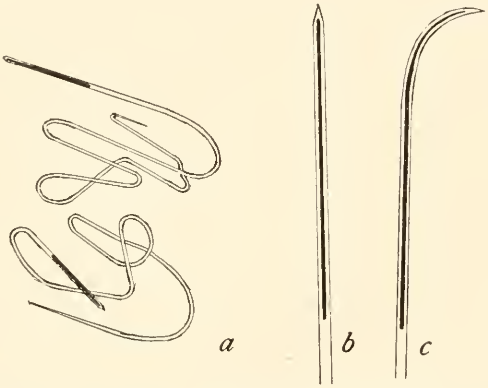

FIG. 1. Mature spermatozoa. (*a*) Entire spermatozoa; (*b*) straight sperm-head, the inner chromatic rod of this type was measured; (*c*) curved sperm-head, a characteristic type not measured.

The author's preparations agree with Paulmier's description of the mature spermatozoön of *Anasa tristis* in having no middle piece distinguishable, so that the tail connects directly with the posterior end of the head. The head has the chromatic elements compacted into a long attenuated rod of nearly constant diameter throughout its entire length. This *chromatic rod* is enveloped by a cytoplasmic capsule. The latter is also attenuated, and has a constant outer diameter. The length of the chromatic rod ranges from 24μ to 36μ and the width from $\frac{1}{3}\mu$ to $\frac{2}{3}\mu$. Although the width is too minute for practical measurement, in general, it seems to be proportional to the length. The cytoplasmic sheath is two to three times as thick as the chromatic rod. It extends 2μ to 3μ anterior to the anterior end of the chromatic rod, and is continuous posteriorly with the sheath of the axial filament. Considerable observation has shown that the frequency curve for chromatic rod length does not differ from the curve for the entire head. Unlike the adult spermatozoa described by Paulmier ('99) and Stevens ('05), the author's preparations show a *distinct region of cytoplasm around the chromatic rod*, and fail to demonstrate that the sheath is entirely, or even mostly "used as food material for the growth of the tail sheath," as Paulmier described it (p. 254; also Figs. 56 and 57). The author's hæmatoxylin preparations show no acrosome or

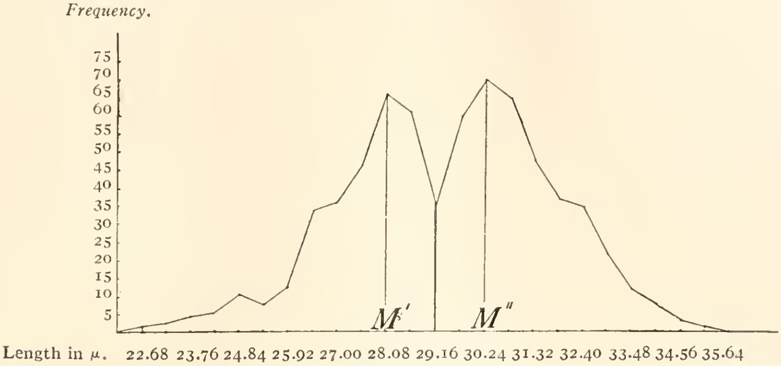

FIG. 2. Bimodal curve of variability of chromatic-rod length for 1r. Greater number of spermatozoa grouped around the upper mode, M″.

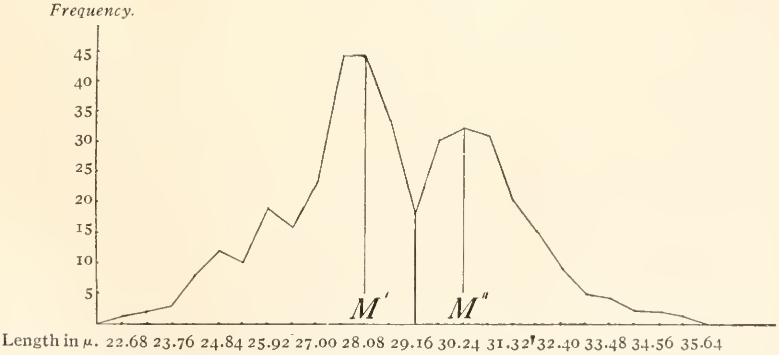

FIG. 3. Bimodal curve of variability of chromatic-rod length for 1l. Greater number of spermatozoa grouped around the lower mode, M′.

centrosome such as Paulmier described for this form. This difference might be claimed to exist because of the different staining capacities of Delafield's hæmatoxylin, used in the author's preparations, and iron-alum hæmatoxylin which Paulmier employed. However, Meek's preparations of the "antepenultimate stage" of *Stenobothrus*, which were stained with the iron-alum hæmatoxylin, show a distinct chromatic rod and sheath, but no acrosome and a diminishing centrosome.

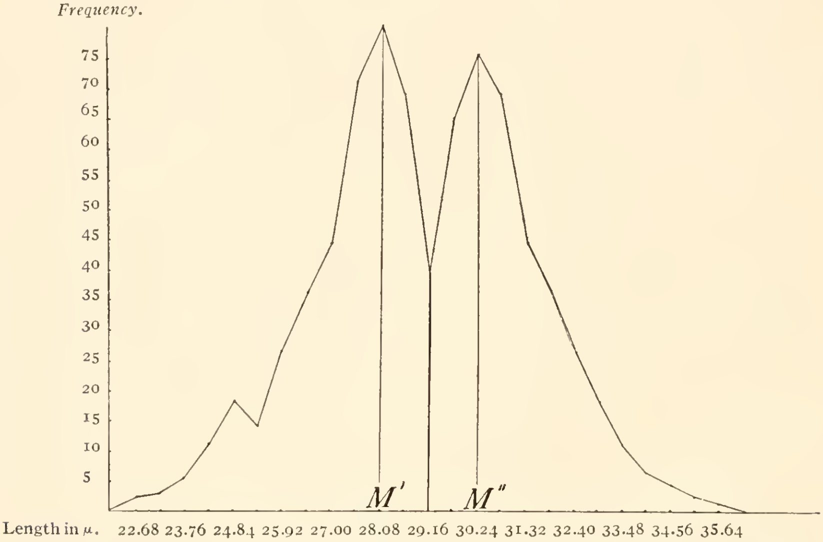

FIG. 4. Bimodal curve of variability of chromatic-rod length for 1r and 1l combined. Number of spermatozoa around both modes approximately equal. Almost bilaterally symmetrical.

Certain spermatozoa of unusually large size were observed. These are the so-called "giant spermatozoa." Such "giant spermatozoa" have been described by Henking ('91), and Wilcox ('95) for various species, and by Paulmier ('99) for *Anasa tristis*. Paulmier described those of "double and quadruple" the normal size, and attributed the phenomena "to the non-completion of one or both of the spermatocyte divisions." The author has observed only the double-sized forms. They are found in about one per cent. of the measurements. These spermatozoa are about one and one fourth times the length of the average normal spermatozoön, and contain about twice the amount of chromatic material of the normal forms. Smith's "giant spermatozoa" of hybrid pigeons, which are twice the normal length, do not appear to belong to this category. In a few cases the author has observed two mature chromatic rods of normal length within a single cytoplasmic membrane. Evidently these were cases where the chromatic elements had divided normally, but where the cytoplasm had failed to divide, so that the two chromatic elements had developed together side by side within the same sheath. It is interesting to note that such "twin" spermatozoa are always of different lengths, varying from 1.5μ to 3μ from each other. "Giant spermatozoa" of this same type have been observed by the author in smear preparations of the housefly, *Musca domestica*.

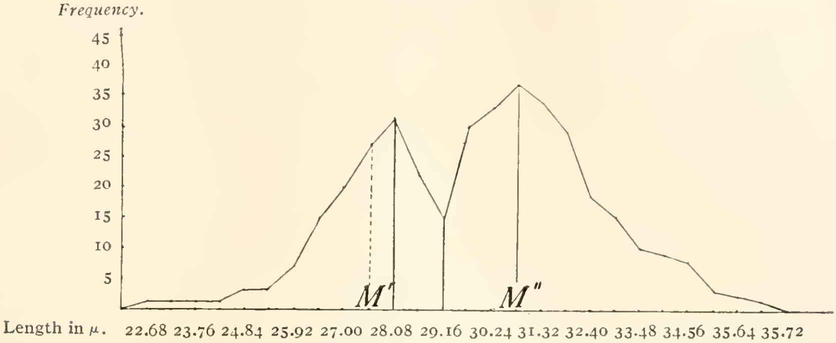

FIG. 5. Bimodal curve of variability of chromatic-rod length for 2r. Greater number of spermatozoa around the upper mode, M″.

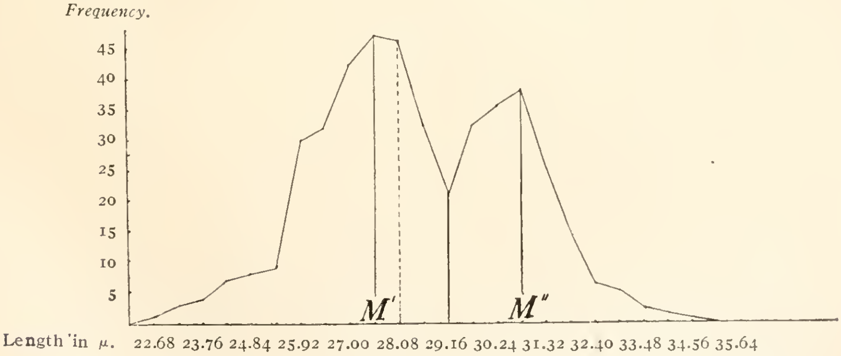

FIG. 6. Bimodal curve of variability of chromatic-rod length for 2l. Greater number of spermatozoa around the lower mode, M′.

The "giant spermatozoa" from the author's preparations had no definite range of variability but blended into the upper reaches of the curve for the normal spermatozoa. The most extreme "giant forms," as well as those that are less extreme, might be considered as wide variants in the frequency distribution of normal forms. If the average normal length is taken, and a "giant spermatozoön" of twice that chromatic volume is computed, then such a form would still fall within the upper reaches of the normal frequency distribution. It is evident, then, that such forms can be considered as "giant spermatozoa" only when an arbitrary upper limit is placed to the normal frequency curve and these wide deviants considered as twice the volume of average spermatozoa.

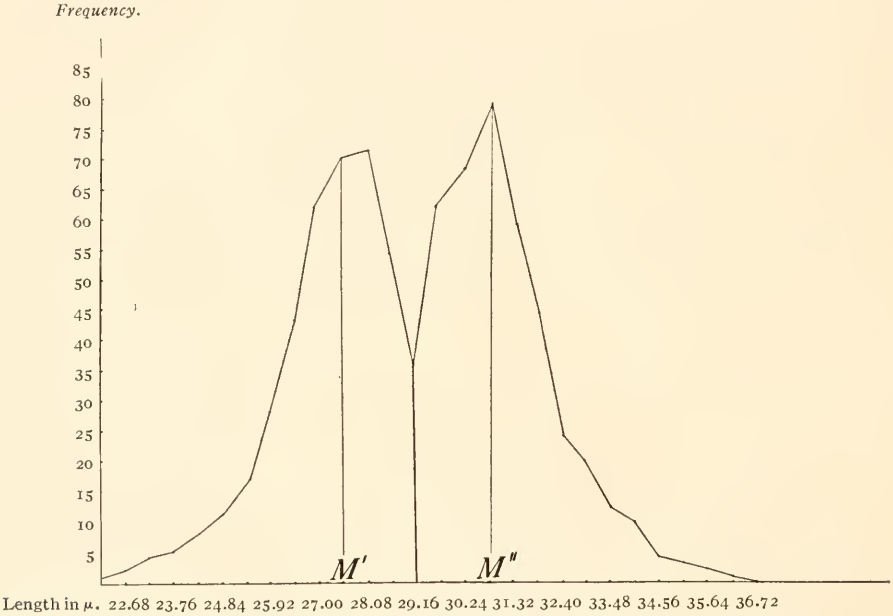

FIG. 7. Bimodal curve of variability of chromatic-rod length for 2r and 2l combined. Number of spermatozoa around both modes approximately equal. Almost bilaterally symmetrical.

(3) *Error Due to Incorrect Measurement.*—It is evident that, for the purpose of measurement, the inner chromatic rod must be well differentiated both anteriorly and posteriorly. Acetocarmine preparation failed in this respect, while the Delafield's

brought it out clearly. Because the posterior limit of the chromatic rod is more easily located than the anterior limit, all measurements were made from the posterior end forward.

Another item to be taken into consideration is that the spermatozoön may not always be in a perfectly horizontal position on the slide, and hence all parts of it may not be in focus at once.

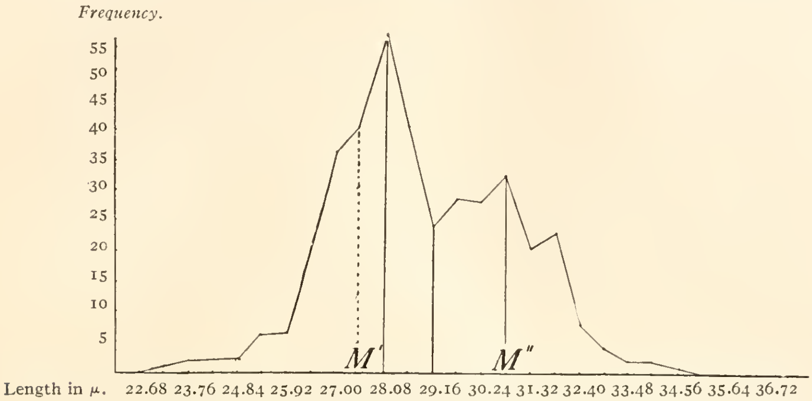

FIG. 8. Bimodal curve of variability of chromatic-rod length for 3r. Greater number of spermatozoa around lower mode, M′.

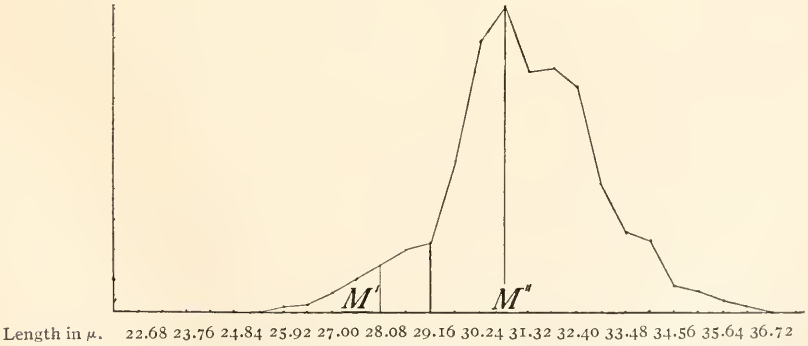

FIG. 9. Unimodal curve of variability of chromatic-rod length for 3l. M″ the only mode.

This may be due to a downward or upward bending or curving of the anterior end of the head, or to a tilting of the entire head, whether curved or straight. To preclude any danger of incorrect measurement all such spermatozoa were recorded separately and only entered in the data with those which were distinctly crooked.

The total of all spermatozoa, curved, crooked and tilted, which were not measured amounted to three to five per cent. of the numbers measured. Had this group been all of a critical length they would not have been sufficient in a single case to change the fundamental character of the curve of variability. An example of such a "curved" sperm-head is shown in Fig. 1, *c*.

A question arose as to how many spermatozoa must be measured before the curve is fairly constant and dependable. It has been observed that with one hundred fifty or two hundred individuals the main features of the curve are outlined; that the curve fluctuates in its details up to three hundred individuals; that four hundred individuals are enough to give a curve of variability which is accurate and dependable. Even a measurement of seven hundred ten individuals, which was made in one case (number 1r), failed to change the character of the curve from that secured for the first four hundred of the seven hundred ten individuals.

In addition to these precautions, several other checks were made in measurement. One preparation, number 1r, was measured three times with three different magnifications; the curves for all three measurements were similar. All measurements were made with the writer's same eye, but were checked by other research students in the laboratory. Finally, only the best of light was used while the measurements were being made.

III. Data Obtained.

The data presented were obtained from eight smear preparations taken from four pairs of testes, fixed and stained by the osmic-Delafield method described above. These preparations are designated as 1 right (1r) and 1 left (1l), 2 right (2r) and 2 left (2l), 3 right (3r) and 3 left (3l), 4 right (4r) and 4 left (4l). Taken as a whole the measurements show very clearly that there is a distinct size dimorphism in the spermatozoa. Individual testes, however, differ considerably from each other in details.

Preparations 1r and 1l.—These spermatozoa when examined in Ringer's solution were very active. From one thousand to two thousand individuals were present on each slide preparation, comprising the total number of spermatozoa from each testis.

With ocular no. 8, seven hundred ten individuals of 1r were measured. This number has been shown to be sufficiently large to plot a reliable frequency curve. Spermatozoa selected were taken as random samples from all parts of the slide. The variability curve is shown in Fig. 2. It shows two distinct modes at the lengths 28.08μ and 30.24μ, with a point of depression midway between at 29.16μ. Not only are the two modes equally distant from the intermediate low point, but the entire curve is approximately bilaterally symmetrical. This same preparation was measured again with a no. 2 ocular and yet again with a no. 12 ocular. In the use of no. 2 ocular five hundred twenty-four individuals were measured; in the use of no. 12 ocular four hundred twenty-nine measurements were recorded. Both of these give curves agreeing closely to the bimodal curve of the first measurement.

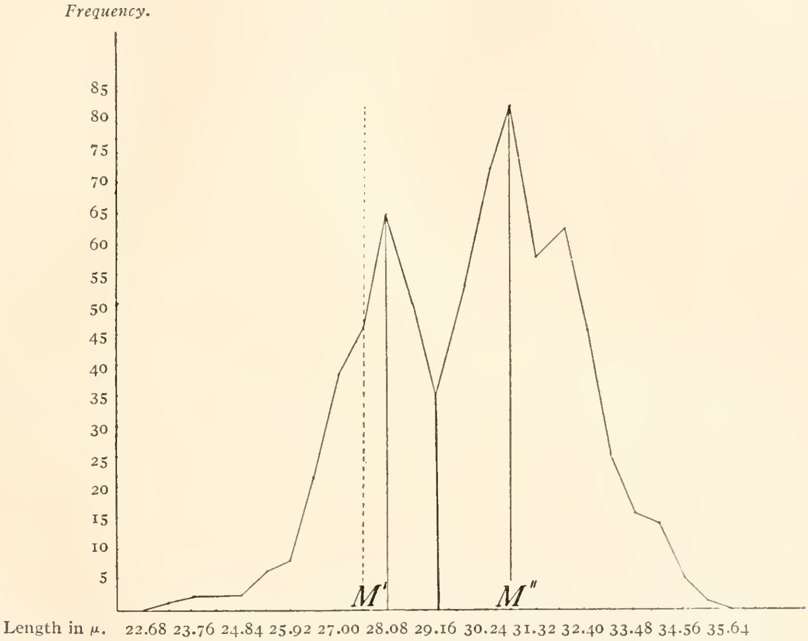

FIG. 10. Bimodal curve of variability of chromatic-rod length for 3r and 3l combined. Number of spermatozoa around upper mode, M'', is greater.

With ocular no. 2, the magnification was hardly sufficient to correctly place the modes. Ocular no. 8 was found most satisfactory and used in all the following measurements. For all these data each measurement was recorded in sequence and later incorporated into a polygon plot. All crooked or curved individuals were entered separately and not used except as a check.

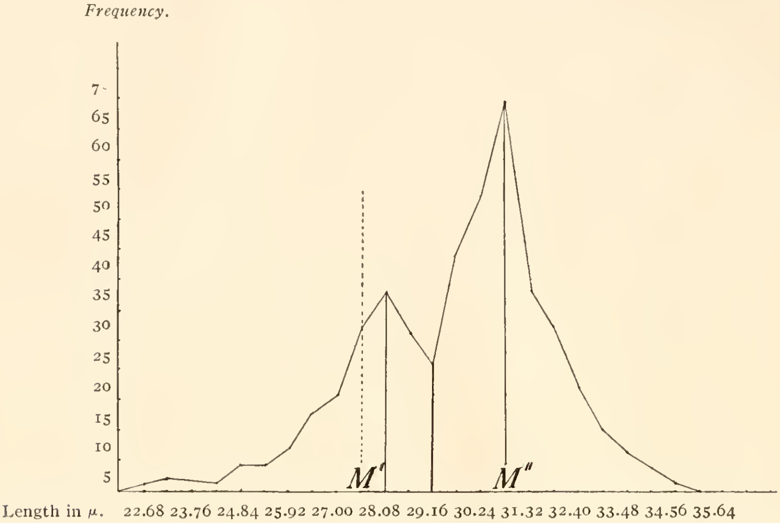

FIG. 11. Bimodal curve of variability of chromatic-rod length for 4r. Greater number of spermatozoa grouped around the upper mode, M″.

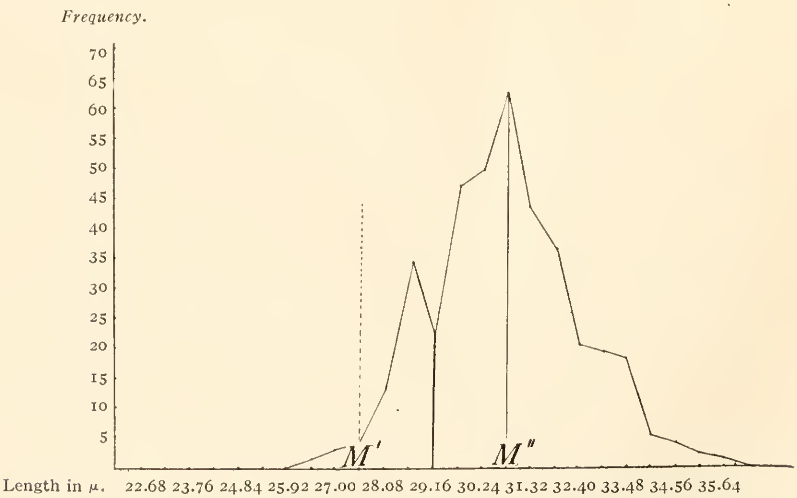

FIG. 12. Bimodal curve of variability of chromatic-rod length for 4l. Greater number of spermatozoa grouped around the upper mode, M″.

They amounted to 5.22 per cent. of the total number measured. Such a per cent. is not sufficient to affect the dimorphic character of the curve, even if it represented spermatozoa all of a critical length, *i. e.*, 29.16μ.

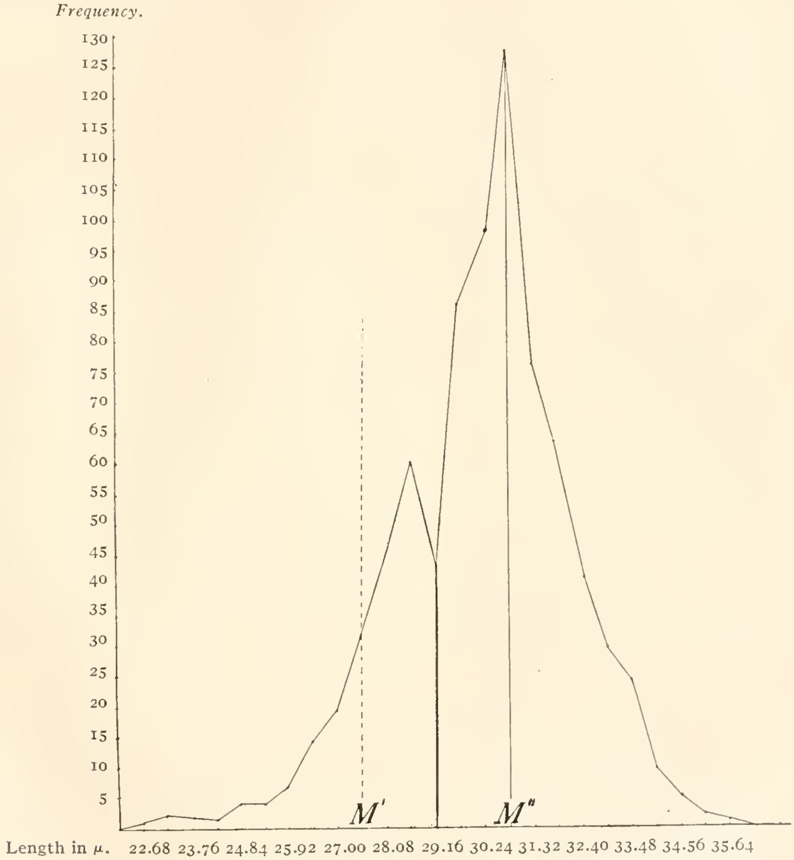

FIG. 13. Bimodal curve of variability of chromatic-rod length for 4r and 4l combined. Number of spermatozoa around upper mode, M″, is greater.

Four hundred individuals from 1l, chosen at random, were measured. This curve also shows a clear dimorphism. It is plotted in Fig. 3.

Preparations 2r and 2l.—Of the right testis 2r, four hundred eighty individuals were measured; of the left testis 2l, four hundred individuals were measured. The plots of these curves, Figs. 5 and 6, show that they are strikingly similar to those of

1r and 1l. It will be noticed, however, that 2r has 30.78μ as a mode of higher frequency, while 2l has 27.54μ as a mode of higher frequency.

Preparations 3r and 3l.—Of 3r only three hundred individuals were measured, because this comprised the total number that was present in the testis. Of 3l five hundred individuals were measured. The curve of 3r is shown in Fig. 8. It is bimodal in character, but with a greater number of individuals grouped around the lower mode. The plot for 3l is shown in Fig. 9. It is unmistakably unimodal in appearance, with the mode at 30.78μ, the length where the upper mode of the preceding bimodal curves occurred. Comparison with the bimodal curves shows plainly that it is the upper half of a bimodal curve, such as that shown in Fig. 2.

Preparations 4r and 4l.—In these preparations four hundred spermatozoa from each testis were measured. 4r is shown in Fig. 11, and 4l in Fig. 12. These are both bimodal and show the dimorphic nature of the spermatozoa, although they differ considerably from the bilaterally symmetrical type.

Preparations made at Woods Hole in August, 1913, from adult males were measured to check the foregoing data. When plotted as a variability curve, they, too, yielded a bimodal curve, with modes at the same lengths as those in the preparations lr and 1l.

IV. Discussion.

The data presented in the previous section show very distinctly the dimorphic character of the spermatozoa of *Anasa tristis*. On the *basis of the chromatic-rod length* they fall into *two classes*, those with an extra amount of chromatin, and those without such an additional amount. Whatever differences and variations there are among the several curves, the differentiation into two groups remains proved. Such minor deviations as occur are probably due to secondary factors not yet discovered, and do not directly affect the bimodal grouping.

This pronounced dimorphic character of the spermatozoa of *Anasa tristis* must bear some fundamental relation to the dimorphism of chromosome number demonstrated by spermatogenesis studies. It is altogether probable that the mature spermatozoa grouped around the higher mode of the curve of variability are those possessing the accessory chromosome, and that those grouped around the lower mode lack this accessory chromosome. The approximate equality of the two groups (see Fig. 2), moreover, strengthens the view that the two groups are produced as spermatids in equal numbers.

However, some of the curves do not show this approximate equality, but have marked digressions from a perfectly bilateral symmetry. They may have been produced as spermatids in equal numbers, and the departure from a bilateral grouping may be due to a difference in nourishment; or this deviation may be due to a slightly earlier ripening of the one group and their ejection from the testis.

Another interesting point brought out by the data is the relation between the right and left testis of the same individual. If the curves of 1r and 1l, 2r and 2l, are examined carefully, they are seen to approach bilateral symmetry even more closely when right and left testes of the same individual are plotted together than when each is plotted separately. Such combinations are produced in Figs. 4 and 7. The former is the combination curve for 1r and 1l; the latter is the combination curve for 2r and 2l. In the combination 1r and 1l, of eight hundred individuals plotted an actual difference of only eight is found between the two sides, or a difference of only one per cent. In the combination 2r and 2l, a difference of only five exists between the two sides of the curve, or a difference of only five eighths of one per cent. On the other hand combination curves for the testes of numbers 3 and 4 show no such close bilaterality. A probable explanation for this discrepancy is offered in the relation between the type of curve and abundance of spermatozoa. In the specimens numbers 1 and 2 there was a great abundance of spermatozoa in each testis. Because four to five hundred individuals were found to be reliable as a basis for a curve of variability, the entire number was not measured, but random samples were taken for measurement. In numbers 3 and 4, on the other hand, a different condition existed. For each of these there were not more than five hundred present in each testis. This abundance or scarcity of spermatozoa may have a very vital connection with the fundamental characters of the curve.

In the cases of most evident bilateral symmetry (in numbers 1r and 1l, 2r and 2l), the preparations were made up early in November, when the spermatozoa were still in abundance. In the cases where the curves do not show this bilateral symmetry (in numbers 3r and 3l, 4r and 4l), such a maximum number of mature individuals was not found. In one testis, at least, in each of these pairs (3r and 4r) the majority of the spermatozoa had been ejected from the testis. This fact was shown by the emptiness of the testis upon first examination. We may therefore provisionally consider the *normal condition* as one in which there is a *maximum number of mature spermatozoa*, and the *bilaterally symmetrical curve of variability* as the *best expression of such a norm*.

It might be assumed that this dimorphism in length is due not to any internal factor which marks out two groups of mature spermatozoa, but represents merely a sudden growth in length whereby the maturing spermatozoa pass over the low intermodal point very rapidly, so that few are "caught" in that place. There are definite reasons why such a theory cannot be accepted. The spermatozoa in the upper and lower reaches of the curve show by their structure that they are equally mature. This is shown not only by their general size and shape, but by their staining reaction which is very different from that of immature spermatozoa. Again, immature forms when they do occur are just as likely to occupy the low intermodal portion of the curve as the mature ones. In fact immature forms have been recorded for such a critical length. Such an objection must be put aside as unjustifiable.

V. SUMMARY.

1. The work presented in this paper has proved the presence of size dimorphism in the adult spermatozoa of *Anasa tristis*. Measurements of the length of the chromatic rod of several hundred spermatozoa from each testis, plotted as a frequency curve, have demonstrated two modes, one between 27.54μ and 28.08μ, and the other between 30.24μ and 30.78μ, and the data offer adequate support for the conclusion that length dimorphism exists.

2. This dimorphism in length of adult spermatozoa probably bears a fundamental relation to the dimorphism in chromosome number revealed by spermatogenesis studies.

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EXPERIMENTS WITH TAPEWORMS.

I. Some Factors Producing Evagination of a Cysticercus.[1]

JOHN W. SCOTT.

During the past winter the opportunity came to me to try a series of experiments upon the bladderworm stage of *Tænia serrata*, one of the common dog tapeworms. The work was made easy because an abundance of material could be secured from the cottontail rabbit in this vicinity. As I have found no record of previous experiments of this kind, it has occurred to me that a brief account of them might be of general interest. One series of experiments in particular I shall here present for consideration. One of the methods used makes it possible to study the process of evagination in the living animal, and to readily secure evaginated cysticerci without the formality of passing them into the intestine of the host.

Tænia serrata is interesting historically from the fact that it was the species first used to demonstrate the characteristic life history of a cestode. Küchenmeister took the bladderworms, known as *Cysticercus pisiformis* Zeder, from the body cavity of hares and rabbits and fed them to dogs. In the course of two to three months he found they transformed and developed into the adult *Tænia serrata*, so that proglottides were detached and lost in the fæces. When the eggs of these forms were fed to hares or rabbits, after the second day of the experiment, minute whitish cysts were discovered in the liver tissue. Subsequently, in about thirty days these parasites left the liver and developed into the full grown *Cysticercus pisiformis*. It is to be noted that this life history involves a parasitism in two entirely different hosts. In general, in the first of these hosts the young tapeworm reaches a stage in development known as a cysticercus, and usually comes to rest in some particular tissue or part of the host's body. But in order to continue development it must be

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[1] Contribution No. 5 from the Zoölogy Laboratory of the Kansas State Agricultural College.

transferred to the intestine of a second or definitive host. If this transfer is not made development stops, after a time death ensues, and degeneration of the young tapeworm takes place. However, if it reaches the intestine of the definitive host, barring accidents, it evaginates the already formed scolex, attaches itself to the mucous membrane by hooks or suckers, grows to maturity, reproduces hermaphroditically, and to complete the life history all that is needed is for the eggs to reach again the particular kind of individual that may serve as an intermediate host. This is of course a matter of common knowledge.

In order to fully understand the experiment it will be best to call to mind something of the structure of a cysticercus and the transformation which takes place when it reaches the intestine. Fig. 1 is a drawing made to show the general structure of a mature cysticercus taken from a half-grown wild rabbit. It is still enclosed within the cyst. When taken from the abdominal cavity a cysticercus consists of a whitish, elongated bladder-like structure filled with a watery fluid; at the smaller end is a dense, compact mass of tissue. Examination shows that this more solid portion of the bladderworm is due to an invagination of the anterior end as shown in Fig. 3. Within the cavity of the invagination is found the apparatus, the scolex, which serves for attachment to the walls of the intestine. In this case the scolex is provided with both hooks and suckers. A few hours after a cyst has been eaten by a dog, a young tapeworm, already transformed, may be taken from the intestine. In this process the invaginated portion evaginates, the scolex is everted, and the bladder digests or is separated and lost in the fæces. Fig. 2 is a drawing made from an evaginated cysticercus that was treated with artificial digestive juices, and Fig. 4 is an enlarged drawing of the scolex of the same.

Now if *Cysticercus pisiformis* is kept in physiological saline (0.7 per cent. NaCl) it never or rarely evaginates. Fifty-five specimens were left in such a solution for three days, in fact until many had died, and at the end of that time only five had evaginated. Besides this occasional evagination, one may produce eversion of the scolex by careful physical manipulation. Braun and Lühe state that this can be readily done in the case of

Cysticercus tenuicollis. "The parasite should be held just below the head-cone in the finger and thumb of one hand, while a regular pressure from within outwards is exerted with the fingers upon the head-cone. The head-cone will lengthen and if the manipulation is repeated several times, it will turn inside out and will hang down as a flat, wrinkled, contractile band upon the surface of the bladder. If pressure is now exerted upon the free end, the head will finally become everted, springing out with a sudden jerk." I have found the process of evagination is much more difficult to produce in the smaller *Cysticercus pisiformis*, and it is not believed that the contraction of stomach or intestine of the host could have any important rôle worthy of consideration in producing the eversion of the scolex.

We must therefore look upon the process of eversion as a response to chemical rather than to physical stimuli. For when fed to a dog practically all cysticerci may evaginate under favorable circumstances. In young, previously uninfected, dogs the percentage rose as high as 90 to 100 per cent. of infection. In old dogs the percentage of infection is not so high. Whether this is due to a sort of immunity dependent upon previous infection, or to some change in the strength of the digestive juices, I am unable to say.

Since it is highly improbable that physical stimuli have any important rôle in producing eversion, it was decided to try the plan of stimulating these animals with artificial digestive juices. The following formulæ were used as a basis in preparing the solutions used in these experiments:

1. For artificial gastric juice:

Water	100.0 parts	
Hydrochloric acid	0.2 "	(dog, 0.3–0.5 part)
Pepsin	0.1 "	

2. For artificial pancreatic juice:

Water	100.0 parts	
Sodium chloride	0.6 "	
Sodium carbonate	0.2 "	(dog 0.4 part)
Pancreatin	0.2 "	

My solutions did not include all of the elements found in the natural product, but the calcium chloride and the potassium phosphate are found in such small amounts in gastric juice that they were considered negligible. That they are not important factors in this experiment was verified by the results. For a similar reason diastase, potassium phosphate and lipase were not included in the artificial pancreatic juice which I used.

Two interesting questions had arisen in this connection: (1) Why is the young parasite not digested in the stomach? (2) What are the factors which influence or produce evagination? The answer to the first question is that as a matter of fact some of the cysticerci probably do meet the fate suggested, and that all react negatively to the gastric juice in such a way that its full effect is hindered or prevented. The answer to the second question will come out as we proceed.

In one of my experiments twenty cysticerci were removed from their cysts and ten were placed in each of two stender dishes. On these was poured a solution consisting of 0.4 per cent. of hydrochloric acid in which was dissolved a small amount of scale pepsin. Upon the addition of this liquid the bladderworms contracted strongly, especially at the invaginated end, and remained perfectly quiet, though they had previously been somewhat restless. The room temperature was about 90° Fahrenheit. Within an hour the bladders were digested enough so that they began to come apart. The cysticerci were left in this solution three and one half hours, and in all this time they showed no signs of activity, though the loose bladders were well digested. The acid solution was then removed and replaced by a solution containing the chief elements of artificial pancreatic juice as described above, leaving out the pancreatin. Within ten seconds several cysticerci had become active, and in a short time a few of them had evaginated. They were restless, however, and very soon all again drew in the scolex. Some pancreatin was now added to the solution; by the time this was dissolved most of the cysticerci were active and in the course of a few minutes ten out of twenty specimens had evaginated, though some of these had hardly completed the process. The dishes were then left over night. The next morning all were completely everted and most of them were relaxed in an excellent condition for preservation or study.

The preceding experiment indicates the probable grave danger that the parasite undergoes in passing through the dog's stomach. It also illustrates the quick response that the bladderworm gives to favorable or to unfavorable surroundings, and the response itself is a reaction to a chemical stimulus. After a considerable number of preliminary experiments in which the main facts of this paper were established, I planned a more complete series, one of which I shall give as typical of the entire set. The earlier experiments were conducted at room temperature.

First, I made up some solutions which contained the principal elements of gastric and pancreatic juices, the formulæ for which have been given. Second, several solutions were prepared containing different combinations of certain of these elements. In most cases the solution had as nearly as possible the same concentration of each component that is found in the natural digestive fluids of the dog. This is shown in the third column of Table I. Equal amounts of these solutions were next put in stender dishes and six well-developed and carefully selected cysticerci were placed in each solution. All stender dishes were then placed in a large water-bath in which the temperature varied less than one degree from 37½° C. I was unable to examine this experiment until four hours and fifty minutes later, and this allowed more time than is ordinarily required for stomach digestion. The general results of this first examination are given in the fourth column of the table. It will be noticed that in lots 2, 3 and 10, in which hydrochloric acid alone was used, there was not a single case of evagination in the eighteen specimens. The same was true for lot 1 where sodium chloride was used. The best result was produced by the artificial pancreatic juice, lot 8, in which five out of six evaginated. The next best results were found in the enzyme solutions, lots 4, 7, and 11, though the reaction to the alkaline solution of sodium carbonate might be considered just as good, lot 6. The dishes were again examined the next morning, 21 hours after the beginning. This allowed considerably longer time than that required for complete digestion, and therefore the results shown in the last column do not come out in such contrast as they do when examined somewhat earlier. For subsequent work has shown that cysticerci when about to die will sometimes evert the scolex under unfavorable conditions.

TABLE I.

TO SHOW THE RESULTS OF AN EXPERIMENT MADE TO DETERMINE THE EFFECTS OF CERTAIN SOLUTIONS UPON THE PROCESS OF EVAGINATION. ALL MATERIAL WAS KEPT IN A WATERBATH AT ABOUT 37½° C. In lots 9, 10 and 11 the solutions were changed after four hours and fifty minutes.

Lot No.	No. Bladderworms Used.	Solution Used.	Result After 4 Hrs. 50 Min.	Result After 21 Hrs.
1	6	0.7% NaCl	None evaginated	1 evaginated
2	6	0.2% HCl	None evaginated	1 evaginated
3	6	0.4% HCl	None evaginated	1 evaginated
4	6	0.2% pepsin sol'n	1 evaginated	2 evaginated
5	6	H_2O—100 pts. HCl—0.4 pt. Pepsin—0.2 pt.	1 evaginated Bladders all digested	1 completely digested Bladder all digested
6	6	0.4 sod. Carb. sol'n	1 evaginated 1 almost evaginated	4 evaginated
7	6	H_2O—100 pts. 0.2% pancreatin	2 evaginated	4 evaginated (slightly acid)
8	6	H_2O—100 pts. NaCl—0.6 pt. Sod. carb.—0.4 pt. Pancreatin—0.2 pt.	5 evaginated	5 evaginated 1 not evaginated (young)
9	6	Sol'n lot 5 followed by Sol'n lot 6	0 evaginated	6 evaginated
10	6	Sol'n lot 3 followed by Sol'n lot 6	0 evaginated	5 evaginated 1 evaginated, partly
11	6	Sol'n lot 4 followed by Sol'n lot 7	2 evaginated	3 evaginated 1 partly evaginated

A study of the table shows the following special results: Lot 1 verifies previous observations, though the salt solution is here raised to body temperature. Therefore, we may conclude that temperature alone appears to have no important influence in producing evagination. From lots 2 and 3 it is also clear that hydrochloric acid interferes with or at least does not help eversion. The pepsin solution slightly aids the process as shown by a comparison of lots 4, 5, and 11. The pancreatin solution is a more favorable medium (lot 7). However, preceding a pancreatin solution by a pepsin solution does not seem to increase the amount of evagination over that when it is used alone (lot 11). Lot 5 shows that artificial gastric juice is harmful to the cysticerci, for it tends to digest them and undoubtedly interferes with evagination (cf. also lot 9). This last effect is due to the acid. Artificial pancreatic juice as used in lot 8 is more effective than either of its elements used separately (cf. lots 1, 6, and 7). But we find that artificial pancreatic juice produces its best results when preceded by treatment with artificial gastric juice. In lot 9 this treatment gave 100 per cent. An almost equally good result was obtained in lot 10 when treatment with an acid (hydrochloric) was followed by an alkali (sodium carbonate).

This experiment or parts of it were repeated several times, but the results were invariably similar in character. Other experiments like these were tried upon the cysticerci of *T. serialis*. The results were so much like those described that there is no need to give details here. The cysticerci of this form are more apt to be digested by the gastric juice, probably due to their smaller size. While much remains to be done in this connection, it is believed that one may safely draw the following general conclusions:

1. Treatment with artificial gastric juice followed by immersion in artificial pancreatic juice furnishes a ready and efficient means of producing the evagination of cysticerci of *T. serrata*. This is an easy method of obtaining material for preservation and for the study of the living parasites. It is best to use the gastric juice in a diluted form if one wishes to preserve the bladders intact.

2. In producing evagination the most important factors in artificial pancreatic juice are the alkali, sodium carbonate, and the extract of pancreatin. But to obtain the best reation to these stimuli previous treatment with hydrochloric acid is required.

3. Treatment with an acid followed by an alkali (lot 10) gives rather better results than treatment with pepsin followed by pancreatin .(lot 11). Hence the sodium carbonate appears to be a more important factor than pancreatin in producing evagination.

4. The cysticerci are not digested in the stomach of the dog because they do not evaginate in a harmful acid medium. The

acid causes them to contract into as dense a mass as possible. This negative response to an inorganic acid is a fundamental and very general characteristic of protoplasm. For example, a very small amount of free acid produces fatal results upon protozoa, developing eggs, spermatozoa, and growing cells in general. "Sour" soils are unfavorable to the growth of most plants. Indeed, this experiment adds one more bit of evidence that one of the primary functions of the acid in the gastric juice is to kill bacteria and other living tissues that reach the stomach.

5. Finally these experiments suggest a way in which one may possibly determine why many tapeworms have a specific definitive host. Not that they have acquired a particular love for that host, but that the special host furnishes the right stimulus at the right time to call forth the proper reaction in the cysticercus. In another paper I have shown that these bladderworms will not produce tapeworms when fed to pigs, and there is one instance on record where a man swallowed five of them, without harmful results. We shall, no doubt, soon be able to explain much of this peculiar and specific behavior of parasites on the basis of a direct response to favorable or unfavorable physical or chemical stimuli. This will bring some of the little understood phenomena of parasitism into line with the brilliant work that has been done in recent years on the behavior of free-living forms.

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EXPLANATION OF PLATE I.

FIG. 1. Diagrammatic drawing of cysticercus of *T. serrata* enclosed in cyst. (× 4.)

FIG. 2. An evaginated cysticercus of about the same size as the preceding. This specimen was treated with diluted artificial gastric juice followed by artificial pancreatic juice. (× 4.)

FIG. 3. Optical section of the anterior end of the cysticercus shown in Fig. 1, with a rather complicated invagination. Drawn with aid of camera lucida. (× 18.)

FIG. 4. Appearance of the scolex after evagination; parts slightly altered in position by pressure of cover-glass. Drawn with aid of camera lucida. (× 46.)

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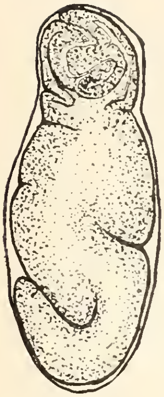

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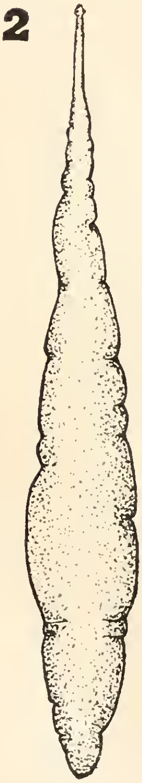

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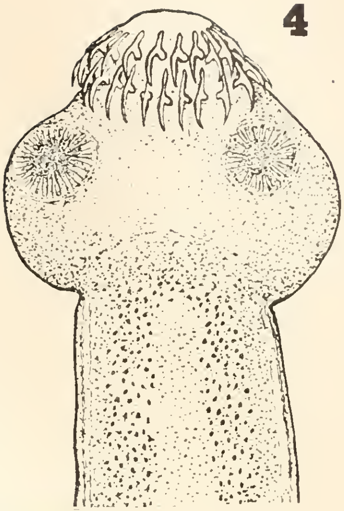

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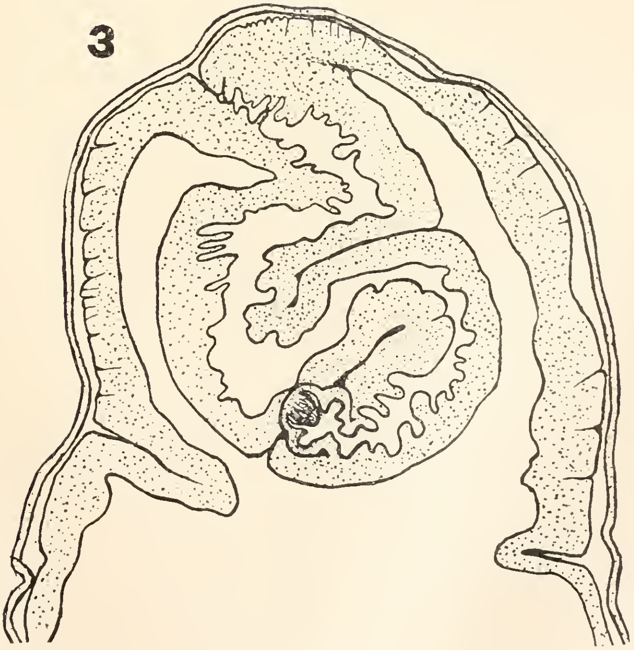

JOHN W. SCOTT.

ON A PECULIAR MONSTROSITY IN A FROG.

GEORGE WAGNER,

Department of Zoölogy, University of Wisconsin.

About three years ago a student, Mr. F. L. Conover, brought me a remarkable specimen of *Rana pipiens*, which he had found among the specimens at the biological laboratory of the Madison High School. The frog was alive. It measured fifty-two millimeters body length and was apparently in full vigor, its death being caused by an overheating of my office at a time when I had to be unexpectedly absent for several days.

The remarkable character of this specimen consists in the presence of three extra limbs extending caudad from the region of the sternum, two on the right and one on the left side. It is very evident that they are attached to an extra basal piece overlying the sternum. All three are more or less imperfect, so that I find it impossible to determine whether they represent arms or legs, or both. Each has four malformed digits, and each shows a thickening indicating the location of the joint between the proximal and distal parts of the limb, the elbow or knee. All are pigmented on the surface away from the frog, with no pigment on the opposite surface. The accompanying photograph (Fig. 2) and radiograph (Fig. 1) of the specimen illustrate better than any verbal description the actual conditions present.

Monstrosities in nature involving the hind legs are not uncommon in the frog, but those involving the fore limbs are apparently rare. The only one known to me is one figured by Sutton ('92, p. 112, Fig. 60), involving the presence of an extra leg on the left side. There is a case in the human being, often quoted, shown in Fig. 3; I have been told about another closely similar case exhibited in this country several years ago, but I have not been able to get exact information concerning it.

Dr. F. R. Lillie kindly called my attention to a very important paper by Tornier (:05) which I would otherwise have overlooked.

Tornier, by an ingeniously chosen cut, separated the upper one quarter or so of the Anlagen of both hind limbs in *Pelobates*. The result was in several cases, at least, the formation of the normal pairs of limbs *plus* two complete extra girdles each with a pair of limbs. Less completely successful experiments resulted in fewer and less fully formed supernumerary parts. But the monstrosities in all cases resembled closely those found in nature, and the close approximation and exposed situation of the Anlagen of the two hind limbs make accidents in nature which may be compared with this artificial "accident" at least not improbable. Tornier's work, it seems to me, gives us a very satisfactory explanation of such forms.

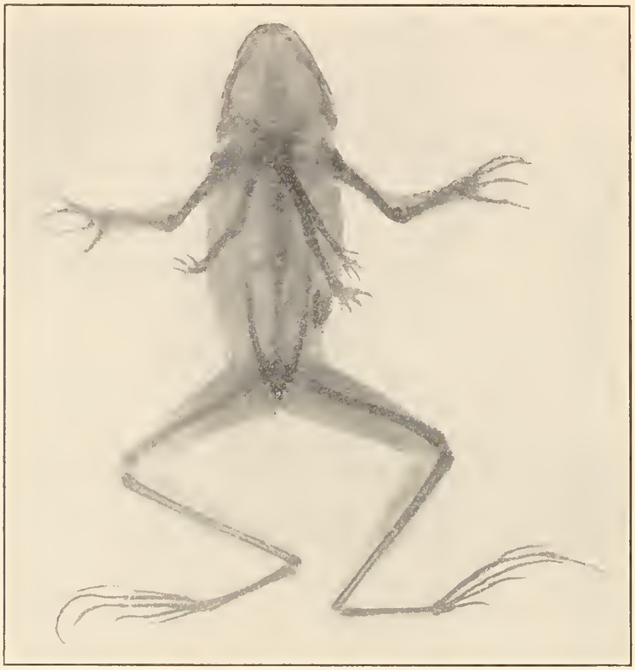

FIG. 1.

Is it equally applicable to the forelimbs? The location of the Anlagen under the opercle makes experimentation here much more difficult, and no such experiments have been carried out, so far as I know. One might expect that by operating on one of the Anlagen a supernumerary arm, such as that of Sutton's case, could be produced. It is an experiment that some skilful operator should attempt to perform. But the Anlagen of the two limbs are here not closely approximated; they are far apart. A simultaneous operation on both is not possible, and an accident in nature equally affecting both is in the highest degree improbable.

It appears much more probable to me that in the frog here described we have a case quite parallel to that in Fig. 3, which is properly designated, according to Schwalbe ('07), as a *Thoracopagus parasiticus*. The generally accepted, and to me very probable, explanation of these forms is that they started with a

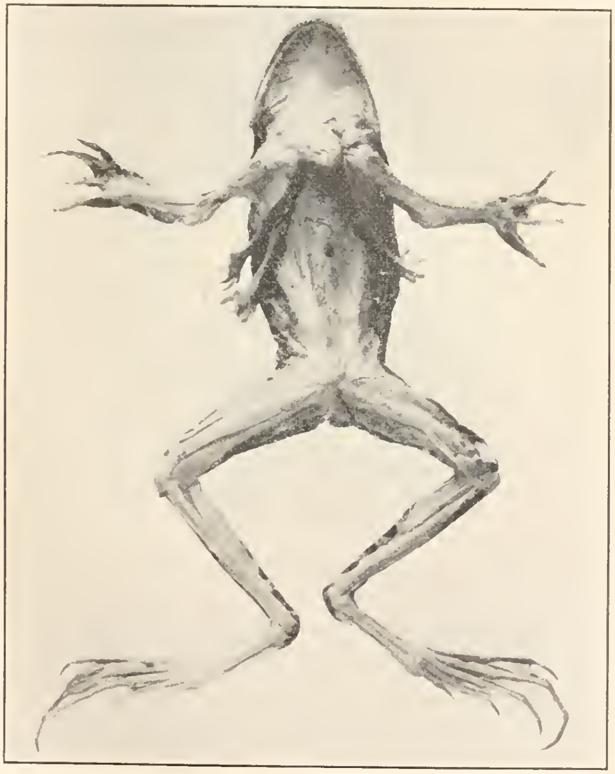

FIG. 2.

monstrosity consisting of two complete embryos united at the breast bone. For some reason or other one gains a start in development. As their circulatory systems are united (as is believed, and in many cases demonstrated) this stronger individual (autosite) soon drives its blood into the other (parasite), the heart of which then degenerates through disuse. This means a less adequate blood supply, atrophy, and a lesser or greater absorption by the stronger individual. It is easy to see that the later in the embryonic period the inequality begins the more extensive in development will the parasite be.

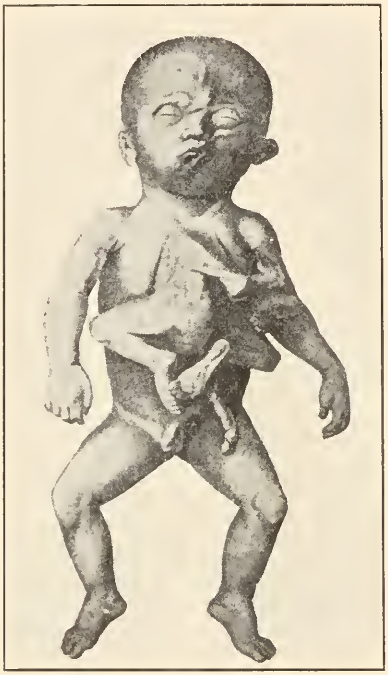

Fig. 3. (After Adami, after Wintersohn.)

I have not attempted to seek all the cases of this sort in man. Approximate parallels of that shown in Fig. 3 must be, to judge from the literature, quite rare. As to the lower vertebrates I have not been able to find a record of any case closely similar to this. It is an interesting fact to note that a frog could grow to maturity with so very material an impediment. It makes one ask just how much of a variation from the normal is needed to enable natural selection to perform its cruel work?

Zoölogical Laboratory,
University of Wisconsin,
July 31, 1913.

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AN EXPLANATION OF THE NON-PRODUCTION OF FERTILIZED EGGS BY ADULT MALE-PRODUCING FEMALES IN A SPECIES OF ASPLANCHNA.

D. D. WHITNEY.

It has been observed by various investigators in the study of certain of the rotifers that in order for a male-producing parthenogenetic female to develop fertilized eggs the female must pair with the male soon after leaving the egg while she is quite young and small. No one has observed the real reason or necessity for this early pairing.

While working with a species of *Asplanchna* in the summer of 1908 at Cold Spring Harbor, New York, and again in the present summer it has been possible to watch the pairing of the two sexes under the microscope. This species is one that has very large individuals, probably as large as any species of rotifer. Both the female and the male are very transparent, thus making it possible to observe all the internal organs, including the ovaries and the eggs of the female, and the organs of the male with the living sperm in the testis. The sperm are very large and can be easily seen with the one third objective. When the male and female pair the two individuals come into contact with each other and the male keeping the head pressed against the body of the female bends the body and assuming the shape of a letter U brings the posterior end into contact also with the body of the female. Then the copulatory organ of the male is forced through the cuticle of the female into the body cavity, like a hypodermic needle, and the sperm passing through it are ejected into the body cavity of the female. The sperm immediately become active and, on account of their large size, can be easily observed swimming around in the body cavity of the female. This hypodermic injection of sperm may be made on any part of the trunk region of the female.

Many observations were made in the pairing of different young females with males. All young females observed were seen to be injected with active sperm from the male. Each female was then placed in a separate watch-glass and allowed to mature and to produce eggs or viviparous young. Some of these young females which were seen to receive living sperm into their body cavities developed parthenogenetic young daughter females and others developed fertilized thick-shelled resting eggs. It has been shown by Maupas,[1] Lauterbaum,[2] Whitney,[3] and Shull[4] that if the young male-producing female pairs with a male thick-shelled fertilized eggs will be produced, but it has never been demonstrated that in pairing the female-producing females also receive sperm into their body cavities in the same manner as do the male-producing females.

Larger and more mature parthenogenetic female-producing females as well as larger and more mature male-producing females were placed with males and many observations made. The male and female individuals come into contact with each other and the male assumes the same position as with the young females. The male makes several attempts to pierce the cuticle of the female with the piercing copulatory organ but is unsuccessful and in some instances sheds the sperm out into the water on the outside of the body of the female. These sperm are active for a few seconds, but soon become inactive and, owing probably to the injurious effects of the water, soon die. This failure of the male to inject sperm into the body cavity of both the adult female-producing female and the adult male-producing female was observed many times and in every case it was a failure. In some instances the sperm were not shed into the water by the male. This seemed to depend more or less upon the condition of the male. If it had been isolated alone for several hours and was young it would shed the sperm out into the water when in contact with the female but if it was old and partly spent it would not shed the sperm out into the water.

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[1] Maupas, M., *C. R. Acad. Sci. Paris*, T. CXI., 1890.

[2] Lauterborn, *Biol. Centralb.*, XVIII., 1898.

[3] Whitney, D. D., *Jour. Exper. Zoöl.*, V., 1907.

[4] Shull, A. F., *Journ. Exper. Zoöl.*, VIII., 1910.

This ability of the male to pierce through the cuticle of young females and the failure to pierce through the cuticle of adult females is due, probably, to the increased thickness or compactness, or both, of the cuticle of the older females. As the females grow and become older their cuticle, which is supposedly of more or less chitinous material, becomes more difficult to penetrate. It is highly probable that if the sperm could be injected into the body cavity of the adult male-producing females that the eggs would be fertilized and thick-shelled resting eggs would be produced.

Males do not seem to be able to discriminate either between each other or young females of either kind or mature females. They attempt to pair with any other individual with which they come into contact. Males have been seen to inject sperm into the body cavities of each other. Many males from a general mixed culture often contain a few free sperm actively swimming around in their body cavities. These sperm have been placed in their body cavities by other males. Thus it happens that more or less of the sperm of a male may be wasted by being injected into either young female-producing females or into other males, or even shed out into the water in attempted impossible copulation with adult females.

SUMMARY.

1. Both kinds of young females, female-producing and male-producing, pair with male individuals and receive active sperm into their body cavities.

2. The young female-producing females having been injected with living sperm by the male mature and produce parthenogenetic daughter-females but the young male-producing females having been injected with living sperm by the male mature and produce thick-shelled fertilized eggs.

3. Male individuals pair and inject active sperm into the body cavities of each other.

4. The mature female-producing females and the mature male-producing females pair with male individuals but do not receive sperm into their body cavities because the copulatory

organ of the male is unable to pierce through the thickened cuticle of the adult female.

5. Much of the sperm of the males may be wasted by being injected into young female-producing females or into other male individuals or by being shed into the water in attempted copulation with adult females.

BIOLOGICAL LABORATORY, WESLEYAN UNIVERSITY,
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BIOLOGICAL BULLETIN

THE RESISTANCE OF FISHES TO DIFFERENT CONCENTRATIONS AND COMBINATIONS OF OXYGEN AND CARBON DIOXIDE.

MORRIS M. WELLS.

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I. Introduction.

In a recent article (Shelford and Allee, '13) entitled "The Reactions of Fishes to Gradients of Dissolved Atmospheric Gases" the authors discussed the physiological effects of gases in solution, on fishes, and presented a table showing the results of some preliminary experiments upon the resistance of fishes to low oxygen. They also report some experiments on the resistance to high concentrations of carbon dioxide.

It is the purpose of this paper to report some further experiments, which have been carried on in the same laboratory, with the purpose of determining what position, varying concentrations of oxygen and carbon dioxide, hold, in the physiology and thus the ecology and economy of fishes. The experiments herein reported were undertaken at the suggestion of Dr. V. E. Shelford, and have been carried on in his laboratory, with the use of part of the same apparatus (gas control) that was used in the experiments referred to above.

II. Plan of Experimentation.

The method followed throughout in these experiments has been, to introduce the fishes into water containing a constant and fatal concentration of oxygen, carbon dioxide, or combination of the two, and then to observe and record the reactions of the fishes, during the time which elapsed between introduction and death. This interval, between introduction and death, was found to be the only time that could be accurately determined for every species used. For this reason, it has been taken as the basis for the data upon which the conclusions of the paper are based. Shelford and Allee give, in their table of resistance to low oxygen, the time consumed between introduction and the turning of the fishes upon their backs. This method was tried in the experiments herein described, but it was found that with the species and conditions used, the "turning time" as they call it, did not, at least in so far as could at this time be determined, represent a definite and comparable time in the succumbing reaction. For example, it was found, that in many instances, the loss of equilibrium was a gradual and not a sudden process, an illustration being the case of the rock bass (*Ambloplites rupestris*). It was found that when two or more species were compared, first, with regard to turning time, and second, with regard to dying time, the comparisons did not show the same relations, in the majority of cases. Furthermore the catfishes and darters often died in a normal upright position, and displayed at no time a reaction that might be taken as the turning point.

It should be stated that Shelford and Allee observed their fishes under conditions which varied considerably from those of these experiments. They confined them in standing water, the temperature of which was not constant; the waste products were allowed to accumulate; and the gas concentrations varied as a result. In the following experiments, the fishes were observed in running water of constant temperature, and very slightly varying gas concentrations.

For the determination of the death point, the best criterion that presented itself was cessation of movement. To make certain that this point might safely be taken as the death point, individuals of different species were, from time to time, removed and placed in fresh tap water, as soon as all movement had ceased. In none of these cases was there recovery or even further movement. However when a fish was removed while the movements were still faintly visible, it usually recovered and became normal once more. The nearness to the death point at which a fish might still be resuscitated varied with the species and with the individual. This variation was not fully determined. It is probable that if errors were made in determining the death point, they were upon the side of exceeding the actual time, for in nearly all cases, a fish was not regarded dead until no movement had been visible for from two to five minutes.

III. Apparatus.

The apparatus used in the experiments consisted of the gas control apparatus, already mentioned,[1] and of three large wide-mouthed bottles, with connections, etc. In brief the gas-control apparatus consists of a series of perforated pans, of boilers, and coolers. The water may be made to pass through the pans into the boilers and through the coolers, in the order named, or it may be turned directly into the boilers and on into the coolers. If gas is to be introduced, it is injected through a gas introducer, between the first and second coolers. In these experiments, the only gas introduced was carbon dioxide. This gas was introduced from the cylinders in which it is purchased. According to Shelford and Allee's analyses, the gas in these cylinders contains 99.4 per cent. carbon dioxide and .6 per cent. nitrogen (different cylinders may vary slightly). Variations in the oxygen concentration were obtained by treatment with the apparatus. The very low concentrations (.1 c.c. per liter) were obtained by boiling the water vigorously in the apparatus, and in order to do this it was necessary to start with the hot tap water of the laboratory. Because it was necessary to use the hot tap water in some experiments it was thought best to use it for all, and this plan was followed except in experiments with high oxygen (10 c.c. per liter). In these cases the cold tap water was used. In either case the temperature at which the water was used was the same, for after the water had passed through the coolers in the apparatus, its temperature was the same as that of the cold water running through the coolers. In these experiments the temperature differed slightly on different days but was constant between 3 and 5 degrees centigrade.

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[1] For full description of gas control apparatus, together with drawings, see Shelford and Allee ('13), p. 214.

By analyses, which are given in Shelford and Allee's paper, it has been shown that there is little chemical difference between the hot and cold tap water of the laboratory. At the time the analyses were made, however, it was found that the tap water after it had passed through the gas control apparatus, showed a slight increase in magnesium content. By later analyses, I have found that this increase was due to the accumulation of magnesium containing scale, in the boilers. To obviate this difference, I have kept the boilers free from any considerable accumulation of scale, and although no further analyses have been made since the beginning of the experiments, I do not think it likely that the effect of the apparatus upon the water has been any greater than the normal daily fluctuations of the water itself, excepting, of course, the differences that have been intentionally produced.

After passing through the gas-control apparatus, the water was led into a 2-liter wide-mouthed bottle (see Fig. 1). The introducing tube led the water to the bottom of this bottle, while the exit tube reached half way to the bottom. Thus all gas bubbles were retained in this first bottle and not allowed to pass into the experimental bottles. From bottle *A*, the water passed into a larger (8-liter) bottle, *B*. This was the first experimental bottle. From bottle *B*, the water was led into still another bottle, *C*, which was the second experimental bottle.

An experiment was conducted as follows. The gas-control apparatus was started and allowed to run from two to four hours, so that the gas concentrations might be regulated and made constant. The time required for this varied with what was to be done to the water. When consecutive tests, five to ten minutes apart, showed the concentrations to be those wanted, and constant, the hose connected with the outlet of the apparatus was slipped over the inlet tube of bottle *A*, the outlet tube of *A* connected with the inlet tube of *B*, etc. After the stream of water had filled all the bottles and had been running through them for some time, during which time the corks of all the bottles had been sealed with modeling clay, it was again tested for concentration and constancy of dissolved gas. The samples were usually collected at the exit tube of bottle *C*. If the tests showed the desired concentration to be present and constant, fishes were quickly introduced, the corks rapidly replaced and resealed, and observations begun. Because of the fact that at the beginning of an experiment one set of fishes usually demanded the entire time of the observer, but one set was introduced at a time. The second set was introduced and observed after the first set had passed through the first and more vigorous stages of the succumbing process.

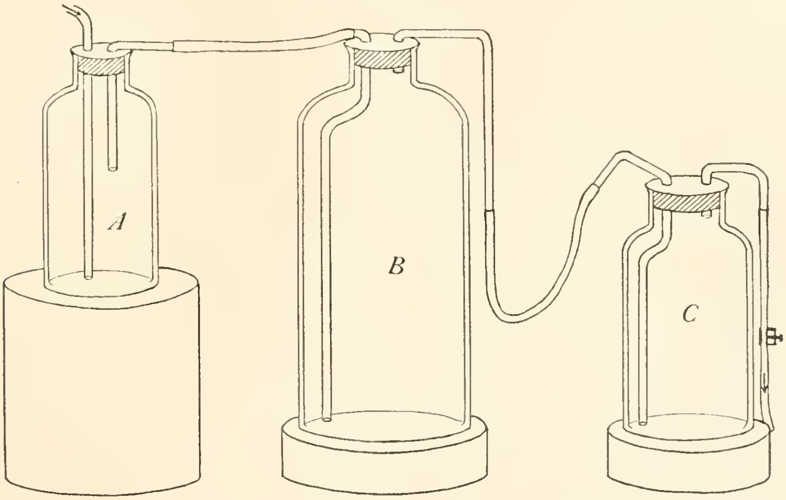

FIG. 1. Showing the arrangement of the catch bottle *A* and the experimental bottles *B* and *C*. In *A* the inlet and outlet tubes are so arranged that bubbles of undissolved gas are retained in the bottle. In *B* and *C* the inlet tubes lead the water to the bottoms of the bottles. The outlet tubes reach only to the bottoms of the corks. This insures thorough mixing of the water and thus conditions throughout the bottles are the same while the water is flowing through.

In order to make certain that the water as it passed through bottle *B* was not affected to a measurable extent, by the presence of the fishes, a number of tests were made of samples collected at the exit tube of bottle *B*. None of these tests showed any measurable difference in gas concentrations, as compared with samples taken at the exit tube of bottle *A*, and it is not probable that the salt content was appreciably affected by the fishes.

The method of analysis that has been used throughout is a slight modification of the standard methods of the Public Health Association.[1] In all cases, the collections of samples were made in narrow-necked bottles, through which the water was allowed to run for several minutes, with the delivery tube reaching to the bottom of the bottle. The bottles were kept corked as much as possible until after all the chemicals had been added. These precautions were essential, for in the case of low oxygen a sample may show 2–3 c.c. per liter, if collected and titrated in an open 250 c.c. graduated cylinder, while if tested as above, it may be found to contain but from .1–.15 c.c. per liter. For the carbon dioxide tests, a special bottle was devised. With this bottle, the cork was removed only to run in the sodium carbonate in titrating. This was always done immediately after the collection was made.

The water flowed through the bottles at the rate of from 500 to 600 c.c. per minute. This changed the water in the larger experimental bottle, once in about 15 min. and in the smaller once in 6 min. In both experimental bottles, the introducing tube led to the bottom, while the exit tube reached just to the under side of the cork. Finally, in order that the temperature of the water might not vary in the experimental bottles, a stream of cold tap water was kept running over each throughout the experiment. The number of fishes used in an experiment varied with their size. A smaller number was usually placed in the smaller bottle. The number in any single experiment varied from 2 to 10 and about 160 were killed, in all. About 25 experiments were performed; the shortest occupied from two to three hours and the longest five to seven days.

The fishes used in the experiments were taken in one of the small creeks near Chicago and were for the most part experimented upon before they had been in the laboratory aquaria for more than two or three days. In no case had they been in captivity for longer than three weeks. In the laboratory they were kept in running tap water and there was practically no mortality. In all some fifteen species were used. In a few instances, few individuals were available and in these cases the data will not be presented unless particularly suggestive.

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[1] See *Jour. Infectious Diseases*, '05, supplement No. 1.

IV. Reactions of the Fishes.

The experiments were of six kinds, this number resulting from the different combinations of gases, those being selected which it seemed would be most likely to give significant results. They were (1) low oxygen (0.1–0.15 c.c. per liter) and high CO_2 (35–50 c.c. per liter); (2) low oxygen and alkaline water (0.5 c.c. N/20 HCl made neutral); (3) low oxygen and medium CO_2 (15–17 c.c. per liter); (4) low oxygen and low CO_2 (about 1 c.c. per liter); (5) medium oxygen (3–4 c.c. per liter) and high CO_2; and (6) high oxygen (8–10 c.c. per liter) and high CO_2.

All the concentrations used are found at times in natural waters. Birge and Juday ('11, pp. 144–169) report concentrations of carbon dioxide for Wisconsin lakes, which vary from about −7 c.c. to nearly +35 c.c. per liter. They also report concentrations of oxygen varying from 0 to 15 c.c. per liter. On page 89 (*l. c.*) they report concentrations of carbon dioxide as high as 47.8 c.c. per liter, in the ground waters of the state. Allee (unpublished) in tests of the water in the series of ponds along the south shore of Lake Michigan has found 40 c.c. per liter of carbon dioxide. In analyses of the water of these same ponds, I have found concentrations of oxygen varying from 1 c.c. to 12 c.c. per liter.

It is probable that the extreme concentrations, as low oxygen (0.1 c.c. per l.) and high carbon dioxide (35–50 c.c. per l.) are not in any sense general conditions, but concentrations not so extreme are of frequent occurrence. Birge and Juday state that conditions of low oxygen and high carbon dioxide may exist for long periods in the deeper waters of the Wisconsin lakes, but the influence of such concentrations upon general fish distribution is probably not so great as that of smaller but more widely distributed concentrations.

The presence of high and low concentrations of carbon dioxide is affected by many factors such as vegetation in the water, character of surrounding soil and incoming water, depth of the water, season of year, daily temperature, animals present, decaying organic matter, rainfall, exposure of the surface of the water to winds. With so many factors affecting the gas concentrations, they will vary greatly even within the same body of water, at any given time. Birge and Juday (*l. c.*) have found this to be especially true in small deep lakes, for different depths. In my own analyses of the waters of the Chicago region, I have found horizontal differences that are also somewhat striking. For instance the lower end of a fifty-foot pool in a small creek may contain 5 c.c. per liter of oxygen less than the water of the upper end. Such variations are common and their influence upon fish distribution must be marked.

In describing the reactions of the different fish species, it will be possible to give a general account which will stand in the main for all the types of experiments. Given in chronological order, the reactions were as follows: Upon introduction into the treated water, the activity of the fishes was usually increased for a short time. This increase was due in part to the handling, but also in part to the stimulation of the water, since fishes introduced in the same manner into normal water were not so active. The greatest increase in activity came with introduction into the low oxygen (0.1–0.15 c.c. per l.) water. The period of activity usually lasted for only 5 to 10 seconds at which time some of the fish occasionally lost their equilibrium (*e. g.*, small-mouth bass), or they sank to the bottom or floated in the water in an upright position. In all of the experiments, and especially in the low oxygen, the opercular movements at once became much more vigorous and continued so up till near the death point. The time period between introduction and the appearance of signs of loss of equilibrium varied with the combination and concentration of the gases present. In general this period varied directly as the lowness of the oxygen concentration or the highness of the carbon dioxide.

The fishes, upon sinking to the bottom, or upon becoming quieter in the water above, rested for a longer or shorter period and then began to "nose" more or less actively about the bottle. Gulping occurred almost from the beginning. This is a normal reaction to some extent but in the experiments the vigor and frequency of the gulping was very noticeable, and gas bubbles were often ejected at the gulps. The fishes now passed through a period of alternating rest and activity. During the early phases of this period, activity was in the ascendency; later rests occupied most of the time. The resting periods lengthened with the gradual loss of equilibrium. The first sign of "staggering" was a falling to a vertical position with the head up. The fish gradually sank to this position and at first made swimming movements which caused it to resume a horizontal position. Later the restoratory movements were not made until the fish came in contact with the bottom. Still later the contact stimulus failed to cause a response and the fish came to rest on the bottom, lying either on its side or on its back. The gill movements were usually still strong and regular. The fish now lay on the bottom, making only occasional swimming movements.

In the low oxygen experiments, the movements of the fishes often became convulsive and uncoördinated before equilibrium was lost, and as time passed, this phenomenon became more and more noticeable. Carbon dioxide, in the concentrations used, did not cause much lack of coördination, but it has been demonstrated that very high CO_2 (100 c.c. or more per l.) will quickly produce this effect. The effect of the carbon dioxide in the concentrations used was that of an anæsthetic which stimulates in small amounts, or at first, but later anæsthetizes. In this connection it was noted that the fishes that were the first to succumb to the carbon dioxide were usually those that displayed the greatest activity.

As the fishes became more and more overcome, the gills developed irregularity in movement; gulps and extra large opercular movements became frequent; later the gills became more or less distended and stiffened, and the movements infrequent. Thus in cases where the gill movements averaged 9–10 in 10 seconds at first, there might now be an average of one or less in the same period. At about this time the gill movements took on a jerky mechanical appearance which continued until they stopped altogether. In nearly all cases, after the gill movements had stopped, life was still indicated by movements of the fins and especially the pectorals, which twitched or waved somewhat regularly for several minutes after the opercular movements had ceased. With the final cessation of movement upon the part of the fins, the fish was considered dead. This final cessation of all movement was often preceded in the low oxygen experiments, by a sudden violent paroxysm, during which, the fish "scooted" about the bottle in a blindly convulsive manner.

V. RESISTANCE OF THE FISHES.

The resistance of the fishes varied with the individual, with the species, and with the size. No reliable data were obtained with regard to individual variation, because of lack of knowledge of the previous history of the animals. Specific and size differences in resistance were however great enough to cover up, to a considerable extent, the unknown individual factor and are to this extent indicatory. With regard to the size (*i. e.*, weight) in the same and different species, it was found that small fishes showed a greater resistance per unit of weight than did the larger ones. At the same time, the larger fishes, because of their excess weight, usually lived longer per individual. These two sets of reactions are clearly shown in the tables which follow.

TABLE I.

SHOWING THE COMPARATIVE RESISTANCE OF LARGE AND SMALL FISHES OF THE SAME SPECIES TO LOW OXYGEN (.1–.15 C.C. PER LITER) AND MEDIUM CARBON DIOXIDE (15–17 C.C. PER LITER).

Species.	No. Fish.		Av. Length in Cm.		Av. Weight in Grams.		Av. Dying Time per Fish in Min.		Av. Dying Time per Gram in Min.	
	Large.	Small.	Large.	Small.	Large.	Small.	Large.	Small.	Large.	Small.
Rock bass (*Ambloplites rupestris*)......	2	2	12.5	5.0	40.7	2.3	371.0	46.5	9.1	20.2
River chub (*Hybopsis kentuckiensis*).....	4	2	10.3	6.5	15.6	3.0	113.5	103.0	7.2	34.3
Blunt-nosed minnow (*Pimephales notatus*)........	2	1	8.5	7.5	6.8	4.3	74.5	67.0	10.9	14.9
Red horse (*Moxostoma aureolum*)......	2	2	9.2	7.2	11.0	3.7	45.0	88.0	4.0	23.8

Table I. gives the results of a few typical experiments in which low oxygen (0.1–0.15 c.c. per l.) and medium carbon dioxide

(15–17 c.c. per l.) were the fatal factors, and illustrates the relative dying order of the large and small individuals of the species used. It will be seen that the larger fishes lived longer per individual, but a shorter time per gram, than the smaller ones. This is shown clearly in the last four columns. That the dying time per individual varies directly, while the dying time per gram varies inversely as the weight of the individual, is still more clearly shown in Table II., which gives this relation for two of the species, namely the rock bass (*Ambloplites rupestris*) and the common shiner (*Notropis cornutus*).

In Table II. the relation holds very closely in the case of the shiner, but seems to break down in the case of the larger individuals of the bass. This apparent variation is to be expected in very large fishes of any species, however, for if the unit weight of the fishes loses power of resistance with increase in age, sooner or later there will come a stage in the life cycle, where the increase in weight, which for a time offsets the decrease in resistance, will diminish and perhaps stop altogether, while the ageing process will go on. Thus the individual resistance curve, which at first rises more or less rapidly, will, at some point, reach its maximum and begin to decline. The fishes will then

TABLE II.

SHOWING THE RESISTANCE OF DIFFERENT-SIZED FISHES OF THE SAME SPECIES TO LOW OXYGEN (.1–.15 C.C. PER LITER) AND HIGH CARBON DIOXIDE (35–50 C.C. PER LITER).

Species.	No. Fish.	Av. Length in Cm.	Av. Wt. in Grams.	Av. Dying Time per Fish in Min.	Av. Dying Time per Gram in Min.
Rock bass					
(*Ambloplites rupestris*)	1	4.4	1.9	20.0	10.5
	1	4.5	2.0	22.0	11.0
	1	5.0	2.1	23.0	10.9
	1	10.5	20.0	103.0	5.1
	1	12.0	31.0	45.0	1.4
	1	14.0	45.0	93	2.1
Common shiner					
(*Notropis cornutus*)...	1	4.5	.6	15	25.0
	1	8.0	4.5	23	5.1
	1	8.5	5.5	37	6.7
	2	9.5	7.0	39	5.5
	1	12.3	17.8	40	2.2
	1	12.5	21.0	45	2.1

be less resistant per individual than smaller fishes of the same species. That such is the case with the rock bass is indicated in the table.

Now if this relation holds in general, the expectation would be, that young fishes of a large species will possess greater powers of resistance than adult fishes of another but smaller species, when the young of the larger and the adults of the smaller species are of the same weight. That such an assumption is tenable is illustrated by nearly all the experiments in which such young and adults occurred. For purpose of illustration, a table showing the results of a series of such experiments is inserted.

Of the species occurring in Table III. the darters and the shiner are small, seldom weighing more than 2 or 3 grams. The red horse is a large species but is easily killed by slight changes in the content of the water (Forbes and Richardson '08, p. 91). Adults of the remaining three species are rather large and resistant.

TABLE III.

SHOWING THE RESISTANCE OF SMALL FISHES TO LOW OXYGEN (.1–.15 C.C. PER LITER) AND LOW CARBON DIOXIDE (1 C.C. PER LITER).

Species.	No. Fish.	Av. Length in Cm	Av. Wt. in Grams.	Approx. Adult Wt. in Grams.	Av. Dying Time per Fish in Min.	Av. Dying Time per Gram in Min.
Darter (*Etheostoma cœruleum*)	3	5.4	2.3	2–3	40	17.3
Red horse (*Moxostoma aureolum*) . . .	2	7.0	3.3	2500.0	52.5	15.9
Shiner (*Notropis atherinoides*)	8	5.0	1.58	1–2	104.0	65.7
Rock bass (*Ambloplites rupestris*) . . .	2	6.0	2.0	300.0	125.5	62.7
Common shiner (*Notropis cornutus*) . .	7	4.8	.79	20.0	195.0	246.7
River chub (*Hybopsis kentuckiensis*) .	2	4.5	.95	30.0	250.0	263.1

A glance at the table will show that although the young of the rock bass, the river chub, and the common shiner, weighed less than the darters, and only in the case of the rock bass more than the shiner (*Notropis atherinoides*), still they proved to be more resistant than the adult darters and shiners, by a considerable margin.

Because of the fact that there was often a break in the increasing weights of the fishes used, and because it was found to be hardly possible to compare species one with another, if the

TABLE IV.

SHOWING THE RESISTANCE OF DIFFERENT SPECIES OF FISHES TO FATAL CONCENTRATIONS AND COMBINATIONS OF OXYGEN AND CARBON DIOXIDE. (FISHES WEIGHING OVER 5 GRAMS AND BEING FROM 8 TO 26 CM. LONG.) LOW O_2 = .1–.15 C.C. PER LITER, MED. O_2 = 2–3 C.C. AND HIGH O_2 = 8–10 C.C. PER LITER. FOR CO_2, LOW = 1 C.C., MED. 15–17 C.C. AND HIGH 35–50 C.C. PER LITER.

Species.	Resistance to												General Average of the Resistances to the Different Factors. The General Average Indicates the Comparative Resistance of the Different Species.	
	1. Low Oxygen and High CO_2.		2. Low O_2 and Alkaline Water.		3. Low Oxygen and Medium CO_2.		4. Low Oxygen and Low CO_2.		5. Medium O_2 and High CO_2.		6 High O_2 and High CO_2.			
	Dying Time per Fish in Minutes.	Dying Time per Gram of Fish in Minutes.	Dying Time per Fish in Minutes.	Dying Time per Gram of Fish in Minutes.	Dying Time per Fish in Minutes.	Dying Time per Gram of Fish in Minutes.	Dying Time per Fish in Minutes.	Dying Time per Gram of Fish in Minutes.	Dying Time per Fish in Minutes.	Dying Time per Gram of Fish in Minutes.	Dying Time per Fish in Minutes.	Dying Time per Gram of Fish in Minutes.	Dying Time per Fish.	Dying Time per Gram of Fish.
Common sucker (*Catostomus commersonii*)	.....	.....	.....	.....	49.3	8.2	350.0	1.8	.....	.....	.....	.....	....	....
Red horse (*Moxostoma aureolum*)	15.0	1.5	20	2.0	45.0	4.0	100.0	14.3	1,865	124	2,072	210	686.1	59.3
Small-mouth bass (*Micropterus dolomieu*)	40.0	3.9	68	8.0	87.0	10.9	70.0	7.2	1,085	54	3,995	790	898.3	147.1
Green sunfish (*Lepomis cyanellus*)	182.0	6.4	210	9.5	215.0	14.3	225.0	11.5	995	90	4,110	326	989.5	76.3
Blunt-nosed minnow (*Pimephales notatus*)	32.0	5.8	50	8.5	74.5	10.8	89.0	10.7	2,902	560	3,995	799	1,190.4	233.4
River chub (*Hybopsis kentuckiensis*)	50.0	4.0	70	5.3	113.5	7.2	68.7	3.1	3,741	366	4,092	511	1,322.5	149.4
Common shiner (*Notropis cornutus*)	40.0	3.4	65	6.3	96.0	10.4	107.0	12.4	4,997	557	5,666	679	1,828.5	228.1
Rock bass (*Ambloplites rupestris*)	88.6	2.8	145	6.3	371.0	9.1	404.2	9.5	8,285	368	8,992	391	3,047.6	115.4
Black bullhead (*Ameiurus melas*)	1,318.0	18.0	.....	.....	.....	.....	1,365.0	18.3	.....	.....	.....	.....	....	....

young were averaged with the adults, the fishes were divided into two groups upon the basis of weight. The dividing line between the groups fell more or less naturally at about 5 grams. Upon calculating the data for the two groups thus formed, it was further found that each group illustrated much the same specific relations, and for this reason but one group, that of the larger or adult fishes, will be discussed. In Table IV. is indicated the resistance of a number of species to different experimental conditions.

Table IV. illustrates the following points: (1) the relative specific resistance of the fishes to the six artificial environments taken singly (vertical columns, except last two); (2) the relative specific resistance of the fishes to the environments taken together (last two vertical columns); (3) the efficiency of each environment as a death-producer for the species used; (4) the antagonistic action of oxygen and carbon dioxide when in the same solution (vertical columns 5 and 6); (5) that an optimum carbon dioxide concentration probably exists for the fishes in question (columns 2 and 4); and (6) that of the concentrations used, the low oxygen was more detrimental to the fishes than the high carbon dioxide (columns 1 and 3).

With regard to the relative resistance of the different species to the environments taken singly, attention is called to the fact that the order varied with the different combinations. This indicates that fish species vary in their resistance to any one factor, the species that is more resistant in one environment being less so in another. The relative resistance of the species to the environments taken as a whole is shown in the last two columns. The order was obtained by averaging the resistances to the single environments. Reading from right to left along nearly any horizontal row of figures will show an increasing resistance of the species to the different environments as arranged in the table. The most fatal combination used was low oxygen (0.1–0.15 c.c. per l.) and high carbon dioxide (35–50 c.c. per l.), and the least fatal, high oxygen (8–10 c.c. per l.) and high carbon dioxide. That this would be the result has already been intimated by Shelford and Allee ('13).

If in the table, column 5 be compared with column 6, it will be seen that the presence of the larger amount of oxygen increased the resistance of the fishes to the high carbon dioxide. Hill and Flack ('10) report this same antagonistic reaction between the two gases and decide from some rather conclusive experiments that the partial pressure of oxygen influences both the higher and the lower limit of carbon dioxide which can be endured by the organism.

A comparison of the second and fourth columns of the table will show that the fishes died more quickly in water that was slightly alkaline than they did in water that was slightly acid. The exact meaning of this difference is not clear. It may be that the alkaline water has some detrimental local effect upon the gills, or it may be that the neutrality mechanism (Henderson, '13) of the fishes is more quickly affected for the worse in the presence of low oxygen, when the water is slightly alkaline than when it is acid. At any rate the results indicate that the fishes have a carbon dioxide optimum. This agrees with Bottazzi's investigations for tissues, as quoted by Jerusalem and Starling ('10). They quote him as saying that "possibly a certain partial pressure of carbon dioxide in the liquid or blood, bathing the tissues, may represent the most favorable condition for the exhibition of the tissues' activity, and that in every tissue it ought to be possible to find an optimum tension of carbon dioxide at which the tissue would do its best work."

A comparison of the different columns in Table IV. will show that the more effective factor in producing death was the low oxygen. One good illustration of this will be noticed in the case of the black bullhead (*Ameiurus melas*) which occurs in columns one and four. In these columns are presented results for two sets of conditions, which differed only in the concentration of the carbon dioxide, the other factor being low oxygen in both cases. It will be seen that the bullhead lived nearly as long in the high carbon dioxide experiment as it did in the low. This indifference of the fishes to variations in carbon dioxide under these conditions is not so clear with the other fishes, but it seems to be generally true that 0.1 c.c. per liter of oxygen will produce death sooner than 50 c.c. per liter of carbon dioxide. It should be noted however (Shelford and Allee, '13) that most fishes detect and react to carbon dioxide even in quite low concentrations quicker than they do to low oxygen. It has already been stated that higher concentrations of carbon dioxide (*e. g.*, 100 c.c. per l.) are more fatal than 0.1 c.c. per liter of oxygen.

VI. General Discussion.

There are a number of questions relating to the physiology of fishes that are touched upon by the results of the foregoing experiments, but no attempt will be made to discuss them at this time, for the data are far too incomplete. There are, however, certain ecological and economic bearings, which may be taken up briefly, with some profitable results.

From the experiments described in this paper, and from those of other workers (Shelford and Allee, '13; Ransom, '66) we may conclude that, in general, the distribution and at times the existence of fishes depend upon two things, namely, (1) the resistance of the fishes to any condition or set of conditions in the environment, that may vary so as to become harmful; and (2) the reaction of the fishes to any such varying condition or set of conditions.

Of these two factors, namely, resistance and reaction, neither can be said to be all-important in any environment, and in most environments the two are inextricably woven together. In the following discussion no attempt will be made to separate the two, but the factor of reaction and behavior will be emphasized over that of resistance, for it seems to me that future investigation must show fully, what previous investigation has already indicated (Shelford, '11; Shelford and Allee, '13*a*), that the reactions of fishes to the conditions of the environment are more vital in determining their distribution and persistence than is their power of resisting adverse conditions.

With regard to behavior and resistance, the fish life cycle can be broken up into four rather distinct periods: (1) the breeding behavior of the adults; (2) the resistance of the eggs and fry; (3) the behavior and resistance of the young fishes; and (4) the resistance and general behavior of the adults.

1. *The Breeding Behavior of the Adults.*—It is generally known that the eggs and fry are stages of relatively low resistance. It

is therefore a matter of much importance that the adults choose a location for depositing the eggs that will enable them to develop normally, and which will prove efficacious for the first stages of growth of the fry.

That the adults make the selection successfully in a majority of cases is undoubtedly true and the fact that the conditions at the breeding grounds may be very different from those of the normal habitat does not seem to interfere with the choice. The conditions necessary for early stages of different species vary widely. Thus the adults of two or more species may live in the same general habitat up to the breeding season when the process of selection of suitable breeding grounds often results in their becoming widely separated. This results in the eggs of the different species being deposited, and the young hatched, under conditions which may differ greatly, but which in most instances prove to be the best for the development of the first stages of each species.

By what process the adults of each species are enabled to select from the great variety of combinations of conditions that combination that is best suited to the development of their own eggs and fry is a matter for investigation. It is probably true that, to a large degree, the choice is a result of reactions, chemical and otherwise, to the factors of the environment. Everman ('98) states that in Louisiana the blue catfish (*Ictalurus furcatus*) and goujon (*Leptos olivaris*) are influenced in their movements by the temperature of the water. During the winter they come farther down the river, where the water is warmest, and in the summer run further up stream or retire to the deeper waters. In some recent experiments with fishes in temperature gradients I have found that many species are very sensitive to slight differences in the temperature of the water, detecting and reacting to differences as small as 1 to 2 degrees C. Gurley ('02) thinks that temperature together with salinity are the factors by which the salmon and other fishes which come from salt into fresh water to breed are enabled to find the freshwater streams.

Green ('09) states that salmon, when coming into fresh water during the breeding season, often spend from 2 to 4 days swimming back and forth in the brackish water before passing on into the streams. Some fishes select certain kinds of bottom for nesting sites, and in doing so probably react to light, temperature and dissolved gases.

What particular factors are most important in the selection of breeding sites is not at present clear; it will probably be found that many factors are acting in most cases, but that some few factors are of such common importance in the breeding reactions of large groups of fishes that they may be used as an index to the breeding behavior of such groups.

In connection with the breeding behavior of fishes there are, besides the general reactions involved in the selection of suitable situations for depositing the eggs, a great number of more specific reactions which are often of the utmost importance in the successful rearing of the new generation. Such reactions are familiar and are illustrated by those of nest-building, aerating the eggs by different devices, guarding the eggs, etc.[1]

2. *Resistance of the Eggs and Fry.*—In spite of the reactions of the adult fishes, which tend to protect the eggs, it is true that of the many thousands that one female may deposit, but very few ever reach the adult stage (Paige, '08) and in most cases the greatest mortality comes at the period when the eggs and fry are developing, (Anthony, R., '08). At this time the relative resistance of the fishes is very low, and many of the eggs probably do not develop because of too great variation in the surrounding conditions; others may develop into abnormal forms which are unable to survive the juvenile period.

Ransom ('66) has shown that a certain concentration of oxygen is necessary in the development of fish eggs; he has also shown that carbonic acid arrests development and may result in the production of abnormal forms which never reach the free swimming stage. Milner ('72) observed that a supply of oxygen was necessary in the successful shipping of eggs, and Loeb ('12) states again the results of some experiments performed upon the eggs of the sea-urchin, in which he found that these eggs can develop only in the presence of free oxygen, that if the oxygen is withdrawn development stops, but begins again if the oxygen is readmitted. He also states that the process of fertilization probably results in a decided increase in the oxidations of the egg. This increase in the oxidations would tend to make the eggs more sensitive to adverse conditions, especially in low oxygen water, and it is very probable that eggs must at times be subjected to severe conditions because of this.

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[1] See citations in Shelford, "Ecological Succession," III., '11.

3 and 4. *Resistance and Reactions of Young and Adult Fishes.*—Of the importance of the resistance powers of fishes in general, in waters where there is no escape from the harmful conditions, there can be no doubt. Also the relative resistance of young and adult fishes is a matter of consequence. In the experiments, described in this paper, it was shown that concentrations of detrimental factors, which are long in affecting adult fishes, may prove fatal to small fishes of the same species, in a comparatively short time. From this it will be seen that, ecologically and economically, the fact that young fishes are more resistant per unit of weight cannot be considered of great importance.

With regard to the resistance of fishes to environmental complexes it is possible to separate environments into two general classes, namely, (1) environments whose conditions are constant or nearly so from season to season and year to year (large bodies of water); and (2) environments where the conditions fluctuate considerably from season to season or from year to year (smaller lakes and streams).

If we consider fishes in their relation to these two types of environments, with resistance as the factor in the foreground, we must look upon the matter of fish existence and distribution as determined by the ability of fishes to withstand the constant conditions in class 1, or the varying conditions in class 2. This then forces us to the conclusion that the matter of fish existence in a given area is one of little flexibility. If, however, we consider the behavior factor as important, it will be seen at once that the matter of fish persistence is now determined by the reactions of the fishes to the two sets of conditions, and becomes at once flexible, because of the fact that the fishes may avoid adverse conditions by moving out of them.

That fishes will react in this way in natural waters is no longer a question of doubt. Both adult and young fishes are sensitive to detrimental concentrations of oxygen and carbon dioxide, as was clearly shown in the experiments described in this paper, and that they will turn back from such concentrations has been shown by Shelford and Allee ('13) and in some later experiments by myself. With regard to the comparative sensitiveness of young and old fishes, Wiegelt ('85) found that young fishes were more sensitive to ammonia than adults, and Shelford and Allee found the same relation to be generally true for their fishes, in the cases of oxygen and carbon dioxide. Such being the case, in natural lakes and streams fishes would generally find a way out of areas of adverse conditions (Green, '09) if the conditions did not appear too rapidly, and the first to become active in seeking escape would be the young fishes.

Many complications suggest themselves in a consideration of the ability of fishes to persist in a given environment. One will be considered as suggestive. What will be the result of planting a species of fishes in an environment, in which by its combined resistance and behavior reactions, the species is able to reproduce, but at a great disadvantage, as for instance high egg and fry mortality?

This question is tied up with the matters of acclimatization, adaptation, etc. There are two main possibilities. First, the adverse conditions may result in the resistance of each succeeding generation being raised; or second, the result may be a lowering of the specific resistance, with each succeeding generation. Lowering the resistance of the species will soon lead to extermination; raising it may result in the species' becoming prolific in waters that at first threatened extermination.

Whether fishes adjust themselves to conditions is not yet clear. There is evidence that they may adjust, in the fact that many lower forms are able to do so. Wood Jones ('12) states that corals of the same species show great variation in structure in different environments; Moore ('97) states that very young oysters will become acclimated to new conditions when older ones will not, and ('08) he further states that "commercial sponges are very susceptible to the influences of the environment and when transplanted from one place to another speedily change in character." Whether fishes will so adjust is a matter for investigation and especially since, so far as is known, fishes are not particularly plastic at any stage.

To determine the efficiency of a given body of water as a fish-producer requires the solution of a large number of problems such as the above, and many of these problems demand that the relative importance of resistance and reaction, in fish distribution and existence, be known. In the discussion thus far, resistance of fishes has been shown to be of considerable importance in fish survival, under certain conditions and at certain stages of development. That fish reaction is the more important factor, however, is indicated largely by one fact, namely, *adverse conditions in natural or artificial fish environments are seldom so general or so sudden in appearance that the fishes cannot escape them, at least to an extent sufficient for survival, by making the proper reactions, and observation and experimentation indicate that fishes, in the majority of cases, make such reactions.* It seems pretty well established that fishes regulate their own distribution, and thus indirectly their existence, largely through their reactions to the environmental factors which they encounter. In other words, fishes seldom put their resistance powers to the test, so long as there is a way out of the disturbing conditions. It follows that resistance of fishes becomes a life and death matter, only when adjustment cannot be made by a behavior reaction, and such situations are rare in the life cycles of most fishes.

On the other hand, fish reactions are an important factor even in the smallest and most enclosed bodies of water, provided the waters are able to support fishes at all. In ponds, for instance, the fishes may be able to withstand the stagnancy of the dry season, by coming to the surface, gulping the surface film, burrowing into the bottom, etc. (Kendall, '10). In some fish environments, vertical differences in conditions are most important in their effect upon fish reactions. In small lakes and streams horizontal conditions are important. In such waters there are areas of vegetation, muddy bottom, sandy or gravelly bottom, ripples, pools, etc. Where such conditions are present there is considerable opportunity for the selection of habitat, upon the part of the fishes, and that they exercise such choice has been demonstrated by Shelford ('11*a*). As a result of the choosing, we find the darters, for example, in the swift-flowing rock-bottomed ripples, while the suckers in general are to be found only in the muddy-bottomed pools. Moreover the two groups of fishes seldom encroach upon one another's environments, even though they be directly adjoining as in streams where ripples and pools alternate.

If fishes are kept to their own environments by rather definite reactions to conditions, then should the conditions begin to change, as for instance, the pool begin to fill with resulting changes in dissolved gases, temperature, light, current, etc., the fishes will move away and will not settle down until they reach, more or less accidentally perhaps, but also by definite reactions at times, another set of conditions, that is similar to that which they formerly inhabited. Furthermore, the conditions might change slowly, or but a little, so that the young fishes and the adults of the more sensitive species would be the first and perhaps the only ones to leave. In this case the result would be the slow, partial and perhaps complete depopulation of the area, provided the adverse conditions prevented the entrance of other fishes from the outside.

Shelford and Allee ('13*a*) found that certain species of fishes will turn back quite definitely from concentrations of carbon dioxide as low as 5–7 c.c. per liter, and from oxygen as high as .7–1 c.c. per liter. This being the case, the above illustration need not be considered as at all hypothetical, for concentrations such as those indicated may be found in certain parts of nearly any system of rivers and lakes. Often the adverse conditions are seasonal in occurrence, or they may appear only when certain factory and sewage wastes are introduced into the waters (Marsh, '07), but whatever the time or cause, the result must be the partial or complete depopulation of the area so long as the adverse conditions continue.

It should be noted in this connection, that small variations (*e. g.*, 5–10 c.c. CO_2 per liter) from the normal, probably in many instances, produce in the long run effects similar to those produced by greater variations (*e. g.*, 25 c.c. CO_2 per liter) in relatively short periods. From the standpoint of ultimate persistence of the fishes, it makes little difference whether they die within an hour, a week, a month, or do not die at all, but merely stop reproducing successfully, the final result must be the same, *i. e.*, the disappearance of the fishes from the area so long as other fishes do not come in from the outside. Furthermore the fishes might continue to reproduce more or less normally, without preventing depopulation, if the adverse conditions caused the young fishes to leave the area.

We can say then that in the long run the fish population of an area will vary directly as the concentration of adverse conditions, or that (1) the moving out of the fishes from the area, and (2) the turning back of outside fishes which tend to enter, will at some concentration of adverse conditions inevitably result in complete depopulation. Furthermore, so long as such reactions are possible, the only part played by resistance will be that the least resistant fishes, since they are usually also more sensitive, will move out or turn back first. In neither case need the matter of dying time be taken into consideration.

Summary.

1. Fishes die from lack of dissolved oxygen or excess of dissolved carbon dioxide.

2. Oxygen in large amounts (10 c.c. per l.) antagonizes the detrimental effect of high carbon dioxide (50 c.c. per l.).

3. The action of detrimental concentrations of carbon dioxide and of oxygen is first to stimulate, and if detrimental enough, to later cause coma.

4. Low oxygen (0.1 c.c. per l.) in alkaline water causes death sooner than low oxygen in slightly acid water. This suggests that the fishes have a CO_2 optimum.

5. The resistance of the fishes to fatal concentrations and combinations of oxygen and carbon dioxide varies with the individual, with the species, and with the size (*i. e.*, weight).

6. Small fishes are more resistant per unit weight than are large ones. This fact is not particularly important ecologically or economically.

7. Ecologically and economically, it does not matter whether the fishes in a given body of water die within a minute, an hour, a week, or do not die at all but merely fail to reproduce successfully; the final result must in any case be the same, *i. e.*, the disappearance of the fishes from the area, unless new stock be constantly added.

8. Fish resistance is important in certain enclosed bodies of water, and at certain periods of the life cycle (egg and fry stages), but the more important factor in fish distribution and survival is the reaction of the fishes to the environment.

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BEHAVIOR OF THE COMMON ROACH (PERIPLANETA ORIENTALIS L.) ON AN OPEN MAZE.

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Technique.

The maze (Fig. 1) used in these experiments was constructed out of a sheet of copper 13.5 inches long and twelve inches wide. Each runway is one inch wide and has neither sides nor top; and between each adjacent runway there is a space one and a half inches wide. This maze contains four blind alleys; two which are straight (Fig. 1, *A*, *B*), one which is L-shaped (Fig. 1, 8 *DE*), and one which bears three culdesacs (Fig. 1, 9 10 11

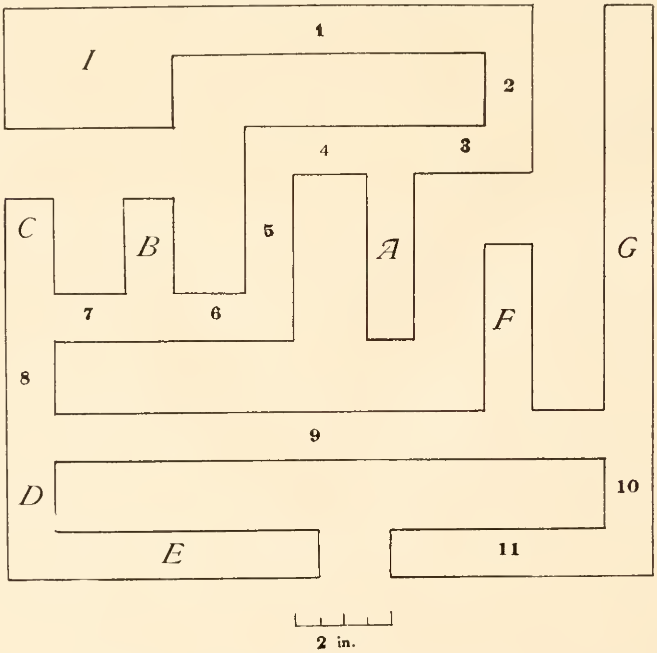

Fig. 1. A diagram of the maze used. *I* is the platform on which the roach is placed. *C* is the point from which the incline departs. The numerals 1–7 indicate the direct passageway to *C*. *A* is blind alley I., *B* is blind alley II., 8 *DE* is blind alley III. and 9 *FG* 10 11 is blind alley IV. of the tables.

FG). When in use the maze is supported, in a horizontal position, by glass pillars which rest in wide pans of water (Fig. 2). These pillars were made by inserting glass stirring rods in the corks of wide-mouth bottles. When thus supported, the maze was about eight inches above the surface of the water which extended beyond it, in all directions, to a distance of eight to twenty inches. To facilitate the taking of accurate notes the parts of the stage were labeled as indicated in Fig. 1.

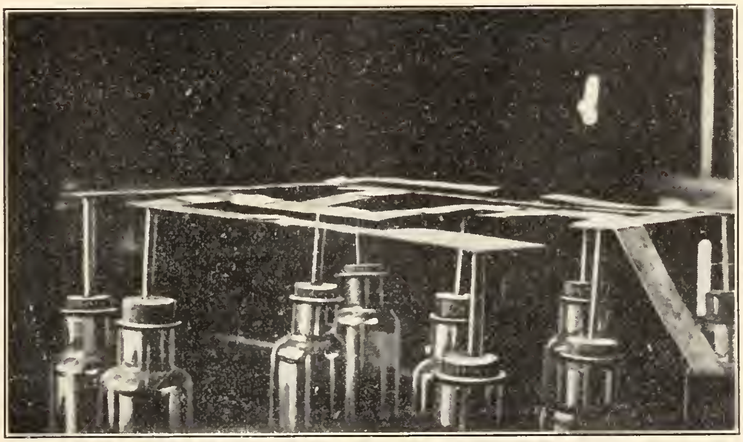

Fig. 2. Photo showing the maze and the upper portions of its support as arranged for use.

These experiments were conducted, in the summer time, in an out-of-doors insectary, the whole north wall of which, except a narrow strip for a door, was constructed of wire netting. The three other walls were without either windows or doors, hence this north opening was the only source of light. Except in the few special cases mentioned in the body of this article, the maze was always arranged with the side *I*1 on the north and parallel to that side of the house.

As originally planned the experiments were to test the ability of the roach to learn to go by the shortest route from the portion of the maze marked "*I*" to a dark box placed at some definite place on the maze. A few preliminary experiments demonstrated that the box did not make a satisfactory goal. Some insects on reaching the box would enter it and remain therein; others would climb up to the top of it, meander around and then descend and go to some other part of the maze. Hence a cardboard incline, extending from some definite part of the maze to the jelly glass that had served as a cage for the roach, was substituted for the dark box.

This same series of experiments taught me that the best place for the incline to leave the maze was the point labeled "*C*." To be of value the incline should be located where the roach, in roaming about the maze, is sure to accidentally discover it. It was found that, in almost all cases, a roach, on reaching the junction of 7, *C* and 8, turned into 8 and, after passing into *D* and often into *E*, retraced its steps and entered *C*; hence an incline placed at *E* would have been too easy a problem. It was noticed that roaches seldom entered 9; hence to place the incline at the extremity of either 11 or *G* would be making the task too difficult. *C* seemed the golden mean.

The roaches used as the subjects of these experiments were isolated in jelly glasses and given an abundance of food. No roach was used in an experiment until it had been in its jelly-glass cage for several days. This was done to get the roach accustomed to confinement and to my presence. The roaches used were females varying in size from young ones ten millimeters in length to full-grown individuals.

The roach to be tested was always placed on *I*. This was done in two different ways. Sometimes the hind leg of a roach was grasped in a pair of forceps and the roach placed on *I*; at others, by means of forceps, the roach was transferred from its glass cage to a narrow cylindrical beaker. This glass was then covered with a small piece of paper and the whole inverted on the maze at *I*. The paper was then removed, and, as soon as the roach quieted down, the cylindrical glass was removed. If a roach ran the maze three times in succession without making an error, it was considered to have solved it. After each trial, the surface and the edges of the maze were painted with alcohol to remove any odor that might have been deposited by the roach.

For convenience, the errors made were grouped under two heads: those which resulted in the roach falling into the water and those made by the roach while remaining on the maze.

Under the first head there are three subdivisions: (1) rushes into the water without attempting to run the maze, (2) falls into the water after the roach had begun to run the maze, and (3) jumps into the water. Under the second head there are two subdivisions: (1) entering blind alleys, and (2) moving in the wrong

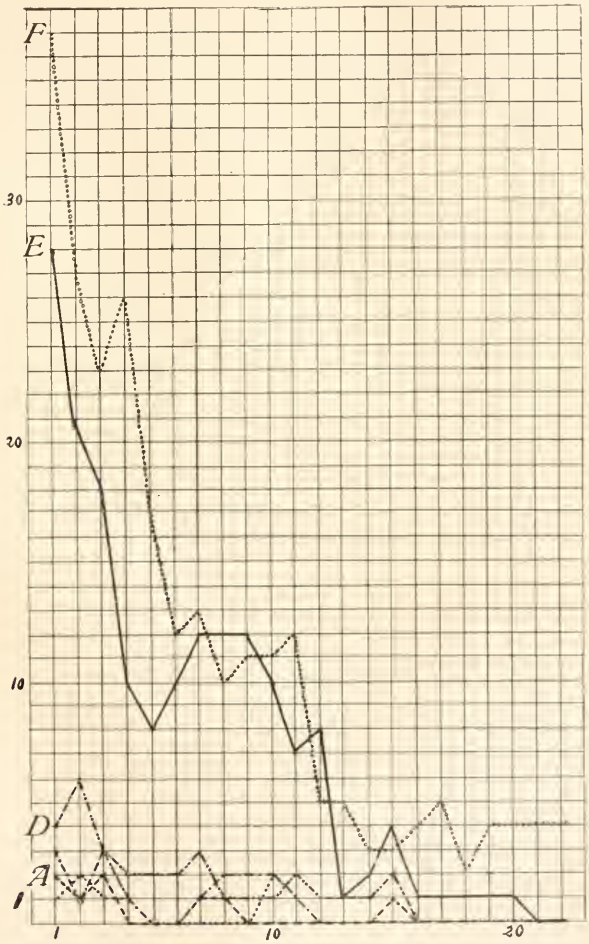

FIG. 3. Learning curves constructed from the average reactions of ten roaches. The spaces from right to left represent successive trials. *A*, the number of times the roach rushed into the water before beginning to run the maze. *B*, the number of times the roach fell into the water after beginning to run the maze. *C*, the number of times the roach jumped into the water. *D*, the total number of times the roach entered blind alleys. *E*, total number of errors made. *F*, total minutes required to run the maze.

direction along the right path. From the time the roach entered a blind alley until it returned to the right path was counted one error. If a roach which was moving in the wrong direction along the right path paused from time to time, each movement after a pause was counted an error. Movements, other than dashes into the water, which were made by a roach on *I* before it had entered the runway 1 were not counted; but once the roach had entered the runway 1, even though it returned to the starting platform *I*, all incorrect movements made by it were counted errors.

In my first experiments I arranged for the successive trials of each roach to come at intervals of several hours. This was done because I had an idea that experiments conducted at short intervals would produce fatigue effects which would vitiate the work. However, I soon found that the best results were obtained where the successive trials came at intervals of about half an hour and were continued throughout the working hours of a day. Towards the end of a long hot day such fatigue effects as a pronounced slowness of movement and the lapsing into errors that the roach had formed the habit of omitting would appear; but, on the whole, the repetition of trials at short intervals was much more satisfactory than the other method of experimenting.

Discussion of the Experiments.

Upon being placed on the maze for the first time, a roach almost invariably rushes off into the water. Upon being replaced on the maze, it usually repeats the performance; some, however, do not rush into the water a second time. Sooner or later it stops rushing into the water and begins to move around in search of some other means of escape. It moves to and fro along the runways, if paths with neither sides nor top may be called runways, enters blind alleys, occasionally falls into the water,[1] makes its toilet one or more times, perhaps engages in a few acrobatic stunts, and finally, by accident, discovers the incline and passes down it to the glass cell that is its home. The first time the roach is placed on the stage, this performance consumes from fifteen to sixty minutes. The next few trials the roach behaves in practically the same manner, but makes fewer and fewer mistakes. Finally, after a prolonged series of trials, in spite of frequent lapses, the mistakes are gradually eliminated and the roach runs the maze, without making any errors, in from one to four minutes. As the roach moves along, the antennæ are waving almost incessantly, as though seeking stimuli. The mistakes are eliminated so gradually that this may be considered a trial and error type of learning, if one may use that expression without predicating the absence of sensations and feelings. Such, in brief, is the behavior of the common roach on the maze; but one is impressed by the variations displayed.

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[1] After it had fallen into the water, the roach was always replaced on the maze at the place from which it had its fall.

These variations in behavior are of two types: differences due to age and modifications due to individuality. The older roaches usually move much more slowly and much more carefully than the younger ones. Roaches from ten to twelve millimeters long usually move so rapidly that they might well be called frisky. The slower gait of the older roaches is not due to feebleness, for they are fleet enough when placed on the floor of a room; but to what, in human beings, we call caution. As a result of this difference in speed, the younger roaches, on their initial trial, usually run the maze much more quickly than the adults; but, in doing so, make many more mistakes.

The variations due to individuality are the ones that are especially impressive. Some roaches, with humped backs, move sedately along in the middle of the runways, pausing at each corner to explore upward, outward and downward with their antennæ; others trot along in the middle of the runways, at first usually falling into the water at each angle, but later slowing up at the corners, exploring with their antennæ and moving onward. Some roaches, with their bodies extended until they are practically flat, drag themselves along so slowly that it taxes the patience of anyone who happens to be watching them; others, moving sometimes slowly and sometimes rapidly, always keep the claws of the legs of one side of the body in contact with the edge of the runway. When such a roach turns a corner it usually, although not invariably, crosses to the opposite side of the runway and clings to the edge with the legs of the corre-

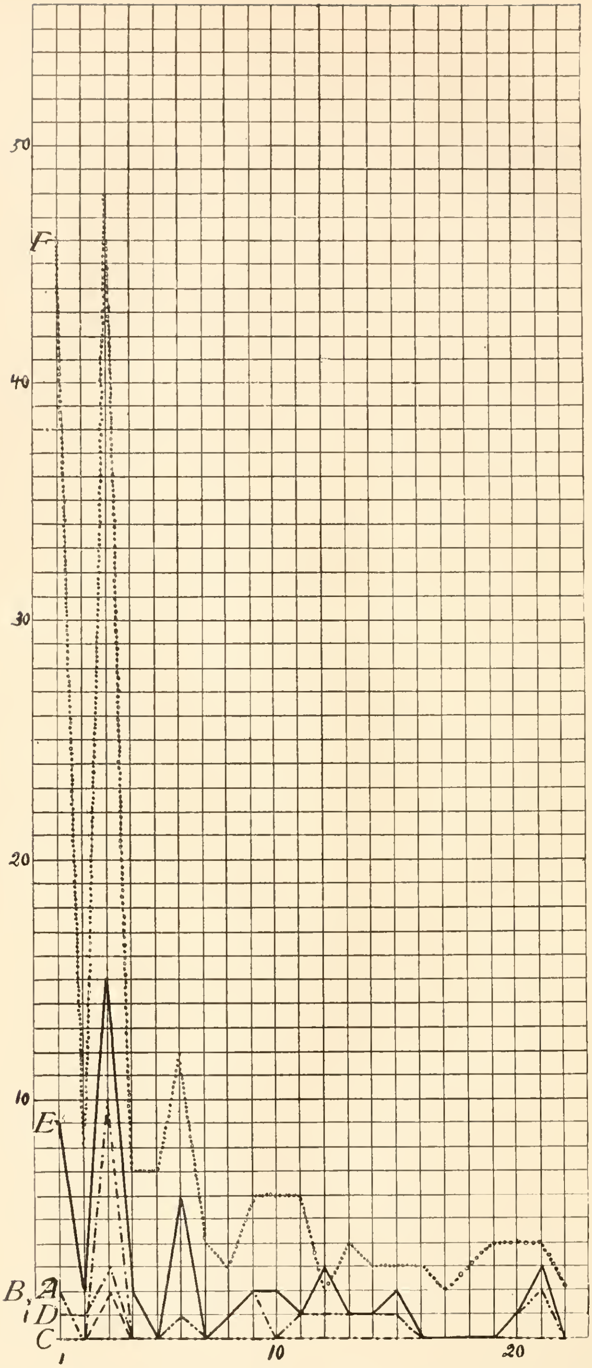

FIG. 4. Learning curves of a female roach 17 mm. long. The spaces from right to left represent successive trials. *A*, the number of times the roach rushed into the water before beginning to run the maze. *B*, the number of times the roach fell into the water after beginning to run the maze. *C*, the number of times the roach jumped into the water. *D*, the total number of times the roach entered blind alleys. *E*, total number of errors made. *F*, total minutes required to run the maze.

sponding side of its body. Some roaches pause at practically every point on the side of the runways and explore upward, outward, and downward with their antennæ, and then, retracing their steps, explore the same territory over and over again; yet other roaches pause only here and there to make explorations. Some roaches, and this is especially true of roaches from ten to fifteen millimeters in length, move along part of the time on top of the maze and part of the time suspended from its edge. Had the maze been constructed of cardboard these same roaches would have made part of their journey suspended from the bottom of the maze. Some roaches pause from time to time to make their toilet; others never once pause for such purposes. Some few roaches, after making several attempts to find an exit from the maze, stop trying and act as though they have given up all hope of succeeding; others, after failing to find a means of escape, attempt to jump to freedom. These jumping roaches were always returned to the maze at the point from which they jumped. After one to many jumps had proved failures, these roaches usually stopped jumping and proceeded to solve the maze in the right way. These variations were not inflexible instinctive responses, for the same roach did not always behave the same way at all trials.

Jumping Activities and Will.

Although this jumping activity results in a plunge into the water, it resembles neither the dashes into the water made by a roach on being placed on the maze for the first time nor the falls into the water by roaches that are trying to run the maze. The roach pauses at the edge of the maze and explores outward and downward with its antennæ. It acts as though it were trying to see something at a distance and then, after a pause, makes what an athlete would call a broad jump. Many roaches displayed this jumping behavior, but some were more prone to jump than others. I experimented with one roach which, on its initial trial, made ten jumps from the maze: usually from a different point each time. This jumping attitude is so characteristic that one can always predict when a roach is likely to jump. I say likely to jump instead of going to jump; because, after a roach has once jumped into the water, the jumping attitude does not always result in a spring. To see a roach, which has learned to avoid rushing off of the maze into the water and which will struggle hard to avoid slipping from the edge of a runway into the water, halt, reach outward and upward with its antennæ, act as though it were trying to see what is beyond, pause and then jump is food for much thought. Have we not here a conflict of impulses and is not the jumping or the refusing to jump the resultant of this conflict? Is not such a resultant what the human psychologists call an act of will? Whenever I behold the jumping behavior, I am impressed with a feeling that the roach is experiencing a conflict of impulses and hence is displaying a will.

In most cases the jumps were made from some one of the outer edges of the maze; but, in a few cases, the jump seemed to be aimed at a definite point. On two occasions a roach jumped and landed on the cork of one of the bottles that supported the maze. At another time, a roach jumped from the runway 2 to blind alley *G* and traveled along that, over zigzaging pathways, to the main trail at *C* and thence to the incline and down it to its cell. On its next trial, the roach attempted to make this same leap; but only its forefeet touched *G* and it fell into the water. On two other occasions I noticed roaches attempt to jump from one runway to another; but they always failed to secure a foothold.

Acrobatic Feats.

The broad jumps described above are not the only acrobatic activities of the roaches that ran the maze. As mentioned above, the roaches frequently moved along suspended from the edge of a runway. With the three feet of one side clinging to the edge of the maze and with the three feet of the other side braced against the under side of the runway, any of the roaches could progress for a short distance, and the young roaches, those from ten to twelve millimeters in length, would run along in this position and return to the upper side at pleasure. In this inverted position, the young roaches could even turn around without returning to the upper side of the runway. Letting go of the edge of the maze with the first and second feet of the off side and catching hold of the edge with the third foot of the

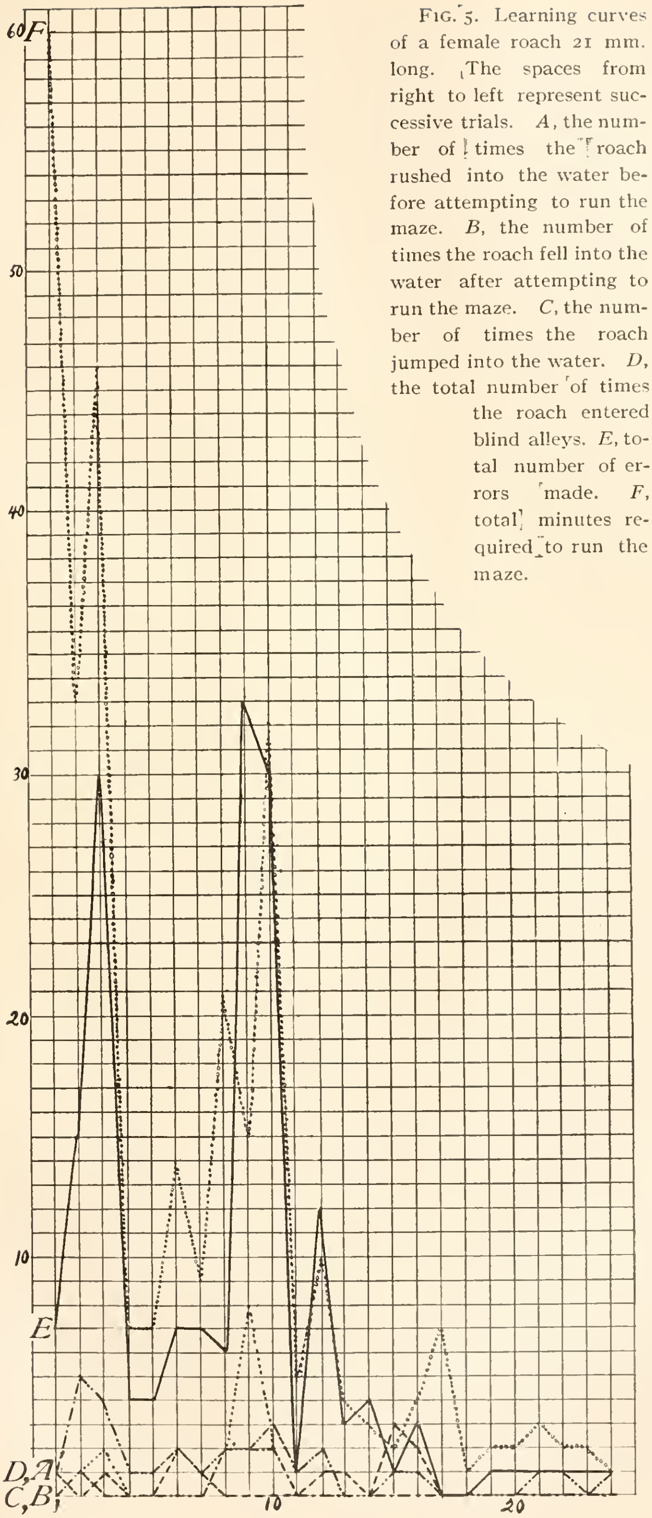

FIG. 5. Learning curves of a female roach 21 mm. long. The spaces from right to left represent successive trials. *A*, the number of times the roach rushed into the water before attempting to run the maze. *B*, the number of times the roach fell into the water after attempting to run the maze. *C*, the number of times the roach jumped into the water. *D*, the total number of times the roach entered blind alleys. *E*, total number of errors made. *F*, total minutes required to run the maze.

inner side, the roach would swing around on the underside of the maze until it could catch hold of the edge with the first and second feet of what had been the inner side. It then would remove the third foot of what had been the outer side and move along in the opposite direction. It was a common thing for roaches ten to twelve millimeters long to rest suspended beneath the maze with the claws of the third pair of legs clinging to the edge of a runway and with the other feet braced against its undersurface.

Frequently a roach was noticed making its toilet while suspended from the maze by one foot. On one occasion I observed a roach, about seventeen millimeters long, which was hanging suspended from the maze by its right third leg, brace itself by bringing the left third leg in front of the other and clinging to the edge of the maze with both feet.

Toilet-Making Habits.

I stated above that frequently movements to solve the maze are interrupted by toilet-making activities. Since most people look upon roaches as nasty things, this toilet-making behavior is a surprisingly interesting instinct. The mouth-parts, the first pair of legs and the third pair of legs are the instruments used by the roach in making its toilet and each has its special work. In cleaning the head and the base of the antennæ the first pair of legs are used in much the same way that a cat uses her forelegs in washing her face. One of the flexed legs is rubbed downward over the head and the base of the antennæ one or more times and then cleaned by the mouth-parts. This may be repeated several times.

As would be naturally expected, the antennæ, which seem to be the most important sense organs of the roach, are cleaned oftener than any other part. If you are standing a short distance from a roach that is resting on the maze, you will notice an antenna suddenly bend downward into the mouth. Slowly it straightens itself while the constantly moving mouth-parts remove the dirt. The next moment the antenna of the other side suddenly bends downward and is treated in the same way. One day a rather keen observer, who was visiting my insectary, was sitting about a yard from a roach that was making her toilet. Suddenly he exclaimed: "What powerful muscles those slender antennæ must contain." Those antennæ do contain muscles which are used in waving them in search of stimuli; but those muscles are far too weak to bend the antennæ into the mouth of the roach. Had that visitor looked a little more keenly he would have seen the roach bow its head, dart one of its forelegs forward, catch in its bend the antenna of the other side and bend it downward to the mouth.

Sometimes, after a plunge into water, the antennæ become so wet that they are held together by capillary attraction and extend forward and upward like the horn of a unicorn. In that position they are out of the range of both of the forelegs. It is interesting to watch the energetic and unavailing efforts of such a roach to clean its antennæ. Standing on its second and third legs with the front part of the body elevated, it moves first one foreleg and then the other after the antennæ in such rapid succession that it resembles a gymnast taking arm exercises.

The palps of the maxillæ and of the labium are cleaned by the mouth-parts; but each palp is flexed into the mouth by its own muscles.

The body assumes a characteristic attitude while the legs and the ventral side of the body are being cleaned by the mouth-parts. Supported by the three legs of one side and by the hind leg of the other, the roach, with the side supported by one leg elevated at the expense of the other, reaches underneath her body and gives her legs and it a good cleaning with her mouth-parts. Again one is reminded of the behavior of a cat making her toilet.

To clean the dorsal surface of the abdomen, the roach uses first one hind leg and then the other as a scraper or brush. With these same legs the cercopods are thoroughly cleaned. In this cleaning process the spines on the legs are quite serviceable.

SENSATIONS.

Plans have been formed to test rather thoroughly the senses of the roach; but, since it will be a long time before the tests can be completed, it is thought best to publish the following preliminary account.

Tactile.—The tactile sense of the roach is remarkably keen. Even a slight jar to one portion of the maze is responded to by a sudden more or less prolonged halt on the part of the roach on a distant point on the maze; and this is true when neither antennæ nor palpi are touching the maze and even when the antennæ have been amputated.

Olfactory.—On the maze itself no conclusive evidence was obtained of the use of this sense. True the antennæ were almost continually waving in space, but it was impossible to determine whether they were seeking olfactory or tactile stimuli. The trap used in capturing the roaches for these experiments was similar to the Graham roach trap described by F. L. Washburn in the *Journal of Economic Entomology* for June, 1913; but I used an eight-ounce bottle instead of an Erlenmeyer flask and no hairs were placed around the apex of the smaller cone. A trap baited with dry oatmeal flakes would capture practically no roaches; but one baited with oatmeal steeped in stale beer invariably captured large numbers. It seems reasonable to assume that it was the sense of smell that enticed the roaches into the trap.

Auditory.—Up to now my notes on the auditory sense are exceptionally non-committal. On the maze roaches seem to pay no attention to sounds produced continuously. For example, a loudly ticking clock was placed two feet from the maze; but to its sounds the roaches made no responses whatever. To certain suddenly produced sounds they responded by halting suddenly, to others they made no response. For example: one day while some tinners were fixing the guttering of a nearby house, to certain noises made by the tin the roaches were quite responsive; but, when, by means of a small bell, I attempted to demonstrate that they respond to suddenly produced sounds, the roaches made absolutely no external responses. By means of a Galton whistle and other methods an attempt is being made to solve the problem; but, up to now, no satisfactory solution has been reached.

Vision.—That the roach possesses vision of some kind is certain, and at times a roach would act as though it were able to distinguish objects at a distance. Recall roaches jumping from one runway to another—a distance of an inch and a half—

and roaches jumping from the maze to the top of a nearby bottle. Yet I could bring a pencil to within a centimeter of the head of a roach without causing a response unless I touched one of the antennæ.

CONCLUSIONS.

1. By arranging the trials at intervals of half an hour, a roach may be taught, within a day, to run the maze.

2. The gradual manner in which it eliminates its errors would cause one to say the roach learns to run the maze by the trial and error method; yet, in so doing, it utilizes sense stimuli. This is evidenced by the careful manner in which it examines (often over and over again) the corners and edges of the maze and the space adjacent thereto.

3. At times the roach acts as though experiencing the emotion the psychologists call will.

4. Although the effects of training persist for a long time, yet the memory of the roach is poor; for after an interval of twelve hours marked lapses were noticed.

5. In its toilet-making activities the behavior of the roach resembles very much the toilet-making activities of the cat.

6. In their behavior on the maze roaches display marked individuality.

Table I.

Successive Trials.	Date.	Number of Hours that Have Elapsed Since the Beginning of the Last Trial.	Errors Due to Movements on the Maze.						Errors which Caused the Roach to Fall into the Water.				Number of Times it Stopped to Make its Toilet.	Total Minutes Required to Run the Maze.
			By Entering Blind Alleys.											
			I. (Fig. 1 *A*.)	II. (Fig. 1, *B*.)	III. (Fig. 1; 8, *D*, *E*.)	IV. (Fig. 1; 9, 10, 11, *F*, *G*.)	Movements in the Right Path, but in the Wrong Direction.	Total.	The Number of Times it Rushed into the Water before Beginning to Run the Maze.	The Number of Times it Fell into the Water After Beginning to Run the Maze.	The Number of Times it Jumped into the Water.	Total.		
1	Aug. 7, 3:55 P.M.	0	0	0	1	0	5	6	1	0	0	1	19	60
2	Aug. 8, 8:02 A.M.	$16\frac{1}{4}$	3	0	1	1	13	18	0	1	1	2	24	33
3	Aug. 9, 6:54 A.M.	23	3	0	0	0	24	27	1	0	2	3	24	46
4	Aug. 10, 8:08 A.M.	$24\frac{1}{4}$	0	0	1	0	3	4	0	0	0	0	0	7
5	Aug. 10, 6:53 P.M.	$10\frac{3}{4}$	1	0	0	0	3	4	0	0	0	0	1	7
6	Aug. 11, 7:52 A.M.	13	1	0	1	0	3	5	2	0	0	2	8	14
7	Aug. 11, 2:05 P.M.	$6\frac{1}{4}$	0	0	1	0	5	6	1	0	0	1	4	9
8	Aug. 11, 3:22 P.M.	$1\frac{1}{4}$	0	0	1	1	0	2	0	2	2	4	10	21
9	Aug. 12, 7:23 A.M.	16	1	1	0	0	15	17	0	8	8	16	4	15
10	Aug. 12, 8:06 A.M.	$\frac{1}{2}$	2	0	1	0	23	26	0	2	2	4	9	32
11	Aug. 12, 9:16 A.M.	$1\frac{1}{4}$	0	0	1	0	0	1	0	0	0	0	0	5
12	Aug. 12, 10:18 A.M.	1	1	1	0	0	7	9	1	0	1	2	3	10
13	Aug. 12, 12:46 P.M.	$2\frac{1}{2}$	0	0	0	0	1	1	1	0	1	2	4	4
14	Aug. 12, 12:54 P.M.	$\frac{1}{4}$	0	0	0	0	4	4	0	0	0	0	2	3
15	Aug. 12, 1:20 P.M.	$\frac{1}{2}$	0	0	1	0	0	1	3	0	0	3	4	2
16	Aug. 12, 1:33 P.M.	$\frac{1}{1}$	0	0	1	0	0	1	2	0	0	2	2	4
17	Aug. 12, 2:29 P.M.	1	0	0	0	0	0	0	0	0	0	0	2	7
18	Aug. 12, 2:58 P.M.	$\frac{1}{2}$	0	0	0	0	0	0	0	0	0	0	4	1
19	Aug. 12, 3:34 P.M.	$\frac{1}{2}$	0	0	0	0	1	1	0	0	0	0	1	2
20	Aug. 12, 4:01 P.M.	$\frac{1}{2}$	0	0	0	0	1	1	0	0	0	0	1	2
21	Aug. 13, 6:04 A.M.	14	0	0	1	0	0	1	0	0	0	0	2	3
22	Aug. 13, 6:45 A.M.	$\frac{3}{4}$	1	0	0	0	0	1	0	0	0	0	1	2
23	Aug. 13, 7:08 A.M.	$\frac{1}{2}$	0	0	0	0	1	1	0	0	0	0	0	2
24	Aug. 13. 8:03 A.M.	1	1	0	0	0	0	1	0	0	0	0	0	1

This is a compilation of the reactions of the first ten roaches examined. Throughout the first part of this series of experiments a long rest was given after each trial.

TABLE II.

			Errors Due to Movements on the Maze.						Errors which Caused the Roach to Fall into the Water.					
			By Entering Blind Alleys.											
Successive Trials.	Date.	Number of Hours that Have Elapsed Since the Beginning of the Last Trial.	I. (Fig. 1, *A.*)	II. (Fig. 1, *B.*)	III. (Fig. 1: 8, *D, E.*)	IV. (Fig. 1: 9, 10, 11, *F, G.*)	Movements in the Right Path, but in the Wrong Direction.	Total.	Number of Times it Rushed into the Water Before Attempting to Run the Maze.	Number of Falls into the Water After Beginning to Run the Maze.	Number of Jumps into the Water.	Total.	Number of Times it Stopped to Make its Toilet.	Total Minutes Required to Run the Maze.
1	Aug. 8, 9:30 A.M.	0	1	0	1	1	0	3	2	10	0	12	13	45
2	Aug. 14, 5:46 A.M.	130¼	1	0	0	0	4	5	2	2	0	4	9	48
3	Aug. 14, 7:04 A.M.	1¼	0	0	1	0	1	2	0	0	0	0	0	8
4	Aug. 14, 7:31 A.M.	½	0	0	0	0	2	2	0	0	0	0	0	7
5	Aug. 14, 8:03 A.M.	½	0	0	0	0	0	0	0	0	0	0	0	7
6	Aug. 14, 8:40 A.M.	½	0	0	1	0	0	1	0	5	0	5	4	12
7	Aug. 14, 9:02 A.M.	¼	0	0	0	0	0	0	0	0	0	0	1	4
8	Aug. 14, 9:37 A.M.	½	0	0	0	0	0	0	0	1	0	1	0	3
9	Aug. 14, 10:14 A.M.	¾	0	0	0	0	0	0	0	2	0	2	0	6
10	Aug. 14, 10:42 A.M.	½	0	0	0	0	2	2	0	0	0	0	3	6
11	Aug. 14, 11:18 A.M.	½	1	0	0	0	0	1	0	0	0	0	0	6
12	Aug. 14, 11:45 A.M.	½	1	0	0	0	2	3	0	0	0	0	0	2
13	Aug. 14, 12:16 P.M.	½	0	0	1	0	0	1	0	0	0	0	2	4
14	Aug. 14, 12:46 P.M.	½	0	0	1	0	0	1	0	0	0	0	2	3
15	Aug. 14, 1:08 P.M.	½	1	0	1	0	0	2	0	0	0	0	2	3
16	Aug. 14, 1:58 P.M.	1	0	0	0	0	0	0	0	0	0	0	1	3
17	Aug. 14, 2:28 P.M.	½	0	0	0	0	0	0	0	0	0	0	0	2
18	Aug. 14, 3:07 P.M.	½	0	0	0	0	0	0	0	0	0	0	6	3
19	Aug. 14, 3:50 P.M.	¾	0	0	0	0	0	0	0	0	0	0	1	4
20	Aug. 14, 4:24 P.M.	½	1	0	0	0	0	1	0	0	0	0	1	4
21	Aug. 15, 5:30 A.M.	13	1	0	0	1	0	2	1	0	0	1	2	4
22	Aug. 15, 5:46 A.M.	¼	0	0	0	0	0	0	0	0	0	0	0	2
23	Aug. 16, 6:35 A.M.	24¾	0	1	0	0	0	1	6	0	0	6	2	12

This table records the reactions of a female roach 21 mm. long. Throughout this series of experiments the rest period between the experiments was short.

Table III.

Successive Trials.	Number of Hours that Have Elapsed Since the Beginning of the Last Trial.	Errors Due to Movements on the Maze.						Errors which Caused the Roach to Fall into the Water.				The Number of Times it Stopped to Make its Toilet.	Total Minutes Required to Run the Maze.
		Due to Entering Blind Alleys.				Movements in the Right Path but in the Wrong Direction.	Total.	The Number of Times it Rushed into the Water Before Beginning to Run the Maze.	The Number of Times it Fell into the Water After Beginning to Run the Maze.	The Number of Times it Jumped into the Water.	Total.		
		I. (Fig. 1, *A*.)	II. (Fig. 1, *B*.)	III. (Fig. 1; 8, *D*, *E*.)	IV. (Fig. 1; 9, 10, 11, *F*, *G*.)								
1	0	2	0	2	0	18	22	2	3	1	6	13	37
2	$18\frac{1}{4}$	2	0	3	1	9	15	1	1	2	4	6	27
3	16	2	0	1	0	10	13	2	2	1	5	7	23
4	$16\frac{1}{2}$	1	0	1	0	6	8	0	1	1	2	7	26
5	$11\frac{1}{2}$	0	0	1	1	6	8	0	0	0	0	4	17
6	5	1	0	1	0	7	9	0	1	0	1	3	12
7	$4\frac{1}{2}$	1	0	2	0	7	10	1	1	0	2	9	13
8	$1\frac{1}{2}$	0	0	1	0	2	3	1	2	0	3	5	10
9	$9\frac{1}{2}$	1	0	0	0	9	10	0	2	0	2	2	11
10	$3\frac{1}{4}$	1	0	0	0	6	7	0	2	2	4	7	11
11	1	1	0	0	1	2	4	0	1	1	2	4	12
12	1	1	0	0	0	6	7	0	0	0	0	2	5
13	$1\frac{1}{4}$	0	0	1	0	0	1	0	0	0	0	2	5
14	1	0	0	1	0	1	2	0	0	0	0	2	3
15	1	1	0	1	0	1	3	1	0	0	1	3	3
16	$\frac{3}{4}$	0	0	0	0	1	1	0	0	0	0	1	4
17	$\frac{3}{4}$	0	0	0	0	1	1	0	0	0	0	1	5
18	$\frac{1}{2}$	0	0	0	0	1	1	0	0	0	0	5	2
19	$\frac{1}{2}$	0	0	0	0	1	1	0	0	0	0	1	4
20	$\frac{1}{2}$	0	0	0	0	1	1	0	0	0	0	2	4
21	$\frac{1}{2}$	0	0	0	0	0	0	0	0	0	0	2	5

This table records the reactions of a young female roach 17 mm. long. Throughout the first part of the series the rest period between two trials was long.

TABLE IV.

Successive Trials.	Date.	Number of Hours that Have Elapsed Since the Beginning of the Last Trial.	Errors Due to Movements on the Maze.						Errors which Caused the Roach to Fall into the Water.				Total Minutes Required to Run the Maze.	The Number of Times it Stopped to Make its Toilet.
			Due to Entering Blind Alleys.				Movements in the Right Path, but in the Wrong Direction.	The Number of Times it Rushed into the Water Before Beginning to Run the Maze.	The Number of Times it Fell into the Water After Beginning to Run the Maze.	The Number of Times it Jumped into the Water.	Total.	The Number of Times it Stopped to Make its Toilet.		
			I. (Fig. 1, *A.*)	II. (Fig. 1, *B.*)	III. (Fig. 1; 8, *D, E.*)	IV. (Fig. 1; 9, 10, 11, *F, G.*)								
1	Aug. 8, 9:13 A.M.	0	0	0	1	0	13	14	1	2	2	5	9	14
2	Aug. 9, 8:19 A.M.	23	1	0	1	1	5	8	1	2	1	4	6	26
3	Aug. 9, 1:43 P.M.	5½	0	0	0	0	0	0	1	0	0	1	2	3
4	Aug. 10, 6:57 A.M.	17¼	1	0	1	0	9	11	0	3	3	6	18	57
5	Aug. 10, 11:18 A.M.	4¼	0	0	1	0	5	6	0	0	0	0	2	21
6	Aug. 10, 2:52 P.M.	3½	1	0	0	0	8	9	0	1	0	1	1	11
7	Aug. 10, 4:26 P.M.	1½	2	0	3	0	17	22	1	0	0	1	23	22
8	Aug. 10, 6:30 P.M.	2	1	0	1	0	6	8	0	0	0	0	3	6
9	Aug. 11, 5:40 A.M.	11¼	1	0	1	0	13	15	0	0	0	0	3	18
10	Aug. 11, 7:00 A.M.	1¼	1	0	1	0	7	9	0	1	1	2	5	12
11	Aug. 11, 8:23 A.M.	1½	3	0	1	1	13	18	2	0	2	4	10	23
12	Aug. 11, 10:54 A.M.	2½	2	0	1	0	5	8	0	0	0	0	5	7
13	Aug. 11, 1:23 P.M.	2½	0	0	1	1	1	3	0	0	0	0	0	7
14	Aug. 11, 2:00 P.M.	½	1	0	1	0	1	3	0	0	0	0	2	2
15	Aug. 11, 2:29 P.M.	½	0	1	1	0	5	7	0	0	0	0	3	5
16	Aug. 11, 3:12 P.M.	¾	1	0	1	0	8	10	0	0	0	0	1	7
17	Aug. 11, 3:46 P.M.	½	0	0	0	0	8	8	0	0	0	0	1	9
18	Aug. 11, 4:37 P.M.	¾	1	0	1	0	5	7	0	0	0	0	2	2
19	Aug. 12, 6:02 A.M.	13½	1	0	1	0	7	9	0	0	0	0	6	22
20	Aug. 12, 6:30 A.M.	½	0	0	0	0	3	3	0	4	0	4	3	12
21	Aug. 12, 6:56 A.M.	½	1	0	1	0	2	4	0	4	0	4	6	13

This table records the reactions of a female roach 10 mm. long which after 21 trials had displayed no ability to learn the maze. It learned to avoid falling into the water; but displayed no ability to avoid making errors on the runways. It finally gave up attempting to learn the maze and tried to jump to freedom. The roach was accidentally killed at the close of the 21st experiment. Its reactions are recorded because it was the most unapt roach that I had.

Anat.
Aug. 30, 1915.

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